

THE AMERICAN JOURNAL
OF PATHOLOGY

THE AMERICAN JOURNAL OF PATHOLOGY

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NUMBER I

RENAL LESIONS IN THE TOXEMIAS OF PREGNANCY *

E. T. BELL, M.D.

(From the Department of Pathology, University of Minnesota, Minneapolis, Minn.)

This paper is based upon a study of the kidneys from 20 cases of toxemia of pregnancy. Particular attention is given to the structural changes in the glomeruli. The effect of pregnancy upon preëxistent nephritis is also discussed.

The clinical manifestations of the toxemias of pregnancy are so varied that it is difficult to arrange them in a logical classification. For the purposes of discussion, however, they may be divided into five groups: (1) typical eclampsia with convulsions; (2) eclampsia without convulsions; (3) preëclampsia; (4) hyperemesis gravidarum, and (5) pregnancy in association with preëxisting renal disease.

I. TYPICAL ECLAMPSIA WITH CONVULSIONS

The characteristic symptoms and signs in this disease are convulsions, hypertension, albuminuria, edema, headache, visual disturbances, nausea and vomiting, vertigo, restlessness, and, especially in fatal cases, coma. These symptoms are by no means all present in every instance, and apparently no single symptom is necessary to establish the diagnosis of eclampsia, but, by definition, typical eclampsia includes only those cases where convulsions are present.

The pathologist is justified in making a diagnosis of eclampsia if he finds hemorrhagic necroses in the liver in a case of pregnancy, but he cannot exclude eclampsia when no necrosis of the liver is found, since this lesion is occasionally absent.

* Received for publication September 1, 1931.

In order to form a basis for the interpretation of the renal lesions which are described in this paper, the various features of eclampsia will be discussed in some detail.

Hauch estimates the *frequency* of eclampsia in Denmark as 1 instance in every 569 births. Seitz gives a rate of about 1 to 500 in Germany. The rate in Baden over a period of fourteen years, according to Gessner, was 1 to 620. Hospital statistics naturally show a greater frequency of eclampsia. Schmechel, at the Dresden Frauenklinik, found 238 instances of eclampsia in 27,340 births (1 to 110); Meyer-Wirz at Zürich, 112 eclampsias in 13,139 births (1 to 117); and Strober at the München Frauenklinik, 336 eclampsias in 46,711 births (1 to 139). Theobald states that eclampsia is very rare in Siam but he gives no statistics.

Eclampsia is more frequent in *primiparae* than in *multiparae*. The usual hospital statistics show 70 to 80 per cent in *primiparae*. Strober, in 336 instances of eclampsia, found 71.1 per cent in *primiparae*, and 28.8 per cent in *multiparae*. Büttner found reports of 179 cases of eclampsia from all sources during a ten-year period in Mecklenburg-Schwerin. Of these 60.2 per cent were *primiparae* and 39.8 per cent *multiparae*. He suggests that the higher percentage of *multiparae* in his statistics is due to the fact that this group is less inclined to go to the clinics than the *primiparae*. On the basis of the relative frequency of *primiparous* and *multiparous* births Büttner calculates that 1 instance of eclampsia occurs to every 220 to 270 *primiparous*, and to every 1100 to 1300 *multiparous* births. The greater tendency to eclampsia in *primiparae* is well established but entirely unexplained.

Period of Gestation: Eclampsia rarely develops before the fifth month of gestation. Fehling, in a survey of 516 cases, found only 5 before the fifth month. A good survey of the literature of early eclampsia is given by Ebeler, who found reports of 55 cases before the fifth month. A few instances occurring in the second and third months are published.

Füth reviewed 56 published reports of early eclampsia. He states that there are only 11 postmortem reports, and that the details of even these are rather meager. Goedecke gives the following distribution of 306 cases of eclampsia, based on the weight of the fetus: 2-3rd month, 1; 5-6th month, 13; 6-7th month, 39; 7-8th month, 53; 8-9th month, 85; 9-10th month, 38; full term, 77 cases. In

an analysis of 384 cases Goedecke found that the first convulsion occurred postpartum in 70 (18.2 per cent). Peckham, in a report of 77 cases, found that 38 began antepartum, 24 intrapartum, and 15 postpartum. Schmechel grouped 238 cases as follows: antepartum 26, intra- and postpartum 184, and postpartum 28. Meyer-Wirz reported 62 antepartum, 32 intrapartum, and 23 postpartum. Strober, in a study of 336 cases of eclampsia, found that 80 began antepartum, 189 intrapartum, and 67 postpartum. It is not clear in any of these statistics how often præclamptic symptoms were present before labor in the group in which the first convulsion occurred postpartum.

It is to be noted that eclampsia develops in the vast majority of instances after the fetus and placenta have attained considerable size. It is estimated that the symptoms are relieved by emptying the uterus in over 50 per cent of the cases. The fact that, in at least 10 per cent, the symptoms first appear postpartum might be explained as a delayed action of the hypothetical toxin, since eclampsia rarely sets in later than twenty-four hours after labor.

Eclampsia may develop as a complication of extrauterine pregnancy (Ebeler), or of ovarian pregnancy (Luniewski).

Wigger reported a case with eclampsia resulting from a hydatiform mole, and gives references to 8 other similar cases. The occurrence of eclampsia in association with moles indicates that if a toxic substance causes eclampsia it is to be sought for in the placenta rather than in the fetus.

Onset: Seitz states that in about 80 per cent of cases of eclampsia præclamptic symptoms are present before the onset of convulsions. Wolff and Zade also find a gradual onset of symptoms in the usual case. But there is a less frequent type with sudden violent onset in which few or no warning symptoms are noted.

Recurrence: Hinselmann, 1924, from a survey of 10,000 cases of eclampsia collected from the literature, concluded that it recurs in about 1.92 per cent of subsequent pregnancies. On this basis, if we accept the incidence of eclampsia as 1 to 500 pregnancies, eclampsia is about ten times as frequent in those who have had a previous attack.

Some recent writers find a rather high incidence of recurrent eclampsia and toxemia. Schmechel, 1929, traced 83 women who had had pregnancies subsequent to eclampsia. Of these, 35 (42 per

cent) had normal pregnancies; 33 (40 per cent) had preëclamptic symptoms; and 15 (18 per cent) had eclampsia. 58 per cent of the women had eclampsia or preëclampsia in a subsequent pregnancy.

Young, 1929, traced 42 women who became pregnant again following an attack of eclampsia. In the 60 gestations which occurred, there were 3 instances of eclampsia, 15 of albuminuria and 6 of abortion, hemorrhage or premature labor. Complications occurred in about 40 per cent, but the recurrence of typical eclampsia was low.

Apparently a woman who has had an attack of eclampsia runs a great risk of developing some form of toxemia in a subsequent pregnancy, although typical eclampsia does not often recur. Some writers attribute the predisposition to toxemia, following an attack of eclampsia, to renal injury, but this view has not been established.

Convulsions: Convulsions are the characteristic feature of typical eclampsia, but the number of convulsions is very variable. Seitz, in a group of 147 cases, found 2 in which only one convulsion occurred. Some patients have tremors but not true convulsions. The convulsive attacks commonly follow the preëclamptic symptoms, but occasionally they precede the other symptoms. The convulsions are commonly attributed to injuries of the central nervous system, and this view is supported by the frequent finding of small hemorrhages, areas of softening and thromboses in the brain.

Convulsions are not a necessary part of the eclamptic picture. This topic will be discussed under "Eclampsia without Convulsions."

Edema: Zangemeister has shown that slight edema of the ankles especially, is found at times in practically all pregnant women. It is apparently due to retention of water by the tissues and not to passive congestion. In general, a moderate edema without albuminuria or other preëclamptic symptoms is not of serious import. Albuminuria is usually present with severe edema. In many instances of eclampsia and preëclampsia, edema is inconspicuous or absent. Edema is apparently not due to renal injury, since it commonly precedes albuminuria. Zangemeister believes that generalized capillary injury with increased permeability is the underlying cause of this form of edema. However, there is no direct evidence of generalized capillary injury. The retention of fluid may be due to injury of the tissues with increased affinity for water.

The Urine: Albumin is found in the urine in practically all instances of eclampsia. Usually it is found in large amounts, but occa-

sionally there is only a trace. In rare instances it first appears late in the illness after the convulsions (Theobald), and then only in small amounts. Cases have also been reported in which no albumin was found at any time either before or after the convulsions (Goedcke, Hiess and Beckmann, Austin, Meyer-Wirz, and Breuning). Albumin may appear in the urine or increase greatly in amount within a short time, so that repeated examinations are necessary to exclude its presence. However, it is well established that convulsions and hypertension may precede albuminuria.

There is usually a moderate oliguria during eclampsia. Sometimes only a little urine is excreted. In the rare form with cortical necrosis of the kidneys there is marked oliguria or anuria.

Erythrocytes are often found in the urine in increased numbers, but they are seldom as numerous as in acute glomerulonephritis. Gross hematuria is not often seen except in association with cortical necrosis. The presence of blood in the urine is, however, not inconsistent with the eclamptic kidney.

Blood Pressure: Hypertension is an almost constant symptom of eclampsia. Schwarz states that he has never seen an instance of eclampsia or preëclampsia without hypertension. Seitz, however, found 13 per cent of his patients with a systolic pressure below 130 mm. Hg. He found the systolic pressure over 150 mm. Hg. in 64 of 98 instances of eclampsia, and 30 of 35 instances of preëclampsia. Hiess and Beckmann reported 13 cases with no elevation of blood pressure on repeated examinations.

Heynemann calls attention to the marked lability of the blood pressure in eclampsia. A temporary hypertension is easily overlooked, especially when the patient is first seen late in the illness. Severe cases, particularly patients in coma, are apt to show a fall of blood pressure. In 8 of 56 cases which he studied carefully, the systolic blood pressure was not above 135 mm. Hg. The usual systolic pressure is 150 to 180 mm. Hg. Heynemann states that he has not seen a patient with pronounced preëclamptic symptoms who did not have hypertension.

Hypertension precedes the convulsions in a vast majority of instances, but not invariably. Seitz cites 3 cases where convulsions preceded the rise of blood pressure.

Kidney Function: The retention of water and sodium chloride in eclampsia is well known. A good discussion of the literature of

nitrogen retention in eclampsia is given by Heynemann. It is clear that the non-protein nitrogen of the blood is nearly always normal or only slightly elevated. Hüssy never found it above 50 mg. Seitz found the non-protein nitrogen above 40 mg. in about half his cases, and above 60 mg. in 10 per cent. Plass reports a slight rise in the non-protein nitrogen after delivery in normal patients, most marked during the first twenty-four hours after delivery. In the late toxemias of pregnancy this postpartum increase is accentuated. Plass is inclined to attribute the nitrogen increase to tissue retention rather than to renal insufficiency. However, as will be emphasized later, the glomerular lesions are sufficiently marked to account for some renal insufficiency.

CASE REPORTS

The following 14 cases are examples of typical eclampsia.

CASE 1. (21-477) A woman apparently 35 to 40 years of age registered at a hotel and asked for a physician, who could not be located that evening. She was found unconscious in her room the next afternoon, November 5. The records of the hospital to which she was taken show high blood pressure and convulsions. A dead fetus 48 cm. long was born without operative interference. Death Nov. 5, 1926, at 8 P.M.

Postmortem Report: No edema; moderate jaundice; no excess fluid in serous cavities; small hemorrhages in the serous membranes; heart, weight 332 gm.; lungs normal; liver, extensive subcapsular hemorrhages, and many small hemorrhages on section.

The kidneys weighed, together, 285 gm. The external surfaces were smooth. On section, the cortices were cloudy and of light yellowish brown color.

Microscopically the glomeruli are slightly enlarged, and the capillaries are very narrow. There is a marked thickening of the capillary basement membrane.

CASE 2. (25-120) Gravid 1. 31 years of age. Admitted Feb. 15, 1925. Duration of pregnancy, about five and a half months. She was seen by a physician early in her pregnancy, and he told her that her kidneys were normal. Edema of the feet from time to time during the five weeks preceding admission. February 1, albumin +; blood pressure 120/80; no edema. February 5, blood pressure 125/92. February 15, admitted in coma; blood pressure 184/90; slight edema of the legs and ankles; faint yellowish tinge to the skin. Urine: albumin + + + +, many hyaline and granular casts. Definite oliguria. Four ounces of urine removed by catheter showed a specific gravity of 1034. Temperature ranged from 99 to 103° F. Spontaneous abortion shortly after admission.

She did not recover from the coma. She had three convulsions while in the hospital. Blood sugar 0.15 per cent; blood urea nitrogen 27 mg.; creatinin 1.5 mg.; van Slyke 46. Death February 18.

Postmortem Report: Edema of ankles; 100 cc. of clear fluid in each pleural cavity; 150 cc. in the pericardial cavity; no ascites; edema of lungs; heart, weight 300 gm.; liver, weight 1900 gm., subcapsular hemorrhages.

The kidneys, together, weighed 300 gm. On section the cortices were very cloudy. The external surfaces were smooth.

Microscopic examination shows anemic glomeruli, small capillaries, marked thickening of the glomerular basement membrane of all glomerular capillaries and some increase of endothelial nuclei in a few tufts.

CASE 3. (25-289) Gravida III. 41 years of age. Admitted April 20, 1925, in the eighth month of pregnancy. She was clear mentally on admission and stated that she had been well until April 17, 1925, when she developed a severe headache. She had one convulsion April 20, before entering the hospital. She stated that she had had convulsions seven years ago during pregnancy. Respirations were 50 per minute, and pulse 120. Blood pressure 165/115. The urine showed a specific gravity of 1037, albumin +++++, no sugar. She went into a convulsion about three hours after reaching the hospital and died a few minutes later.

Postmortem Report: No edema; no jaundice; moderate excess of fluid in the serous cavities; heart, weight 390 gm.; moderate left ventricular hypertrophy, no endocarditis, normal valves; edema of lungs; liver, weight 2180 gm., fatty, many hemorrhagic necroses; uterus contained a 2660 gm. fetus; multiple adenomas of thyroid.

The kidneys weighed 200 gm. and 190 gm. respectively. The external surfaces were smooth, the cortices cloudy.

Microscopically the kidneys show changes of unusual interest. The most impressive feature is tubular atrophy which involves over four-fifths of all the tubules. The atrophy varies from a slight decrease in size to almost complete disappearance of the tubules. The glomeruli associated with normal-sized tubules show only the acute changes of eclampsia, that is, thickening of the capillary basement membrane, but all the other glomeruli show various degrees of obliteration. Fully 20 per cent of the glomeruli are completely hyalinized and their tubules have almost disappeared (Fig. 2). The most common glomerular lesion is a focal hyalinization (Figs. 3 and 4), which is somewhat similar to healed embolic glomerulonephritis. The

tubules associated with these partly obliterated glomeruli are atrophic, but not so small as those belonging to completely hyalinized glomeruli. There is some hyaline degeneration of the afferent arterioles, but this is not marked and is not responsible for the glomerular changes.

The lesion does not resemble ordinary glomerulonephritis. The hyalinization is focal, there are no epithelial crescents and no leucocytes. There are also no enlarged glomeruli with endothelial proliferation such as one finds in chronic glomerulonephritis.

There is no basis for a diagnosis of healed embolic glomerulonephritis since the heart valves and mural endocardium are entirely normal.

All transitions are easily found between capillaries with thickening of the capillary basement membrane and those that are completely hyalinized. The process consists simply in progressive increase in the thickness of the basement membrane.

The hyaline glomerular lesions are in all probability the result of the eclampsia seven years before death.

Unfortunately there was no clinical study during the seven-year interval between the attacks of eclampsia, and consequently we do not know if this was clinically chronic renal disease. However, the left ventricular hypertrophy strongly suggests that hypertension was present, and the extensive atrophy of the tubules of the kidney makes it certain that some renal insufficiency was present.

CASE 4. (25-771) Gravidia I. 40 years of age. Unmarried. Admitted Sept. 21, 1925, about eight and a half months pregnant. Nausea and vomiting throughout pregnancy, some headache for several months, edema of feet and hands past two or three weeks, almost total blindness past two or three days. September 21, blood pressure 226/150; scanty urine with heavy albuminuria; hemoglobin 75 per cent. September 22, vomiting; blood pressure 184/110; edema of legs, hands and eyelids; eyegrounds edematous; scanty urine which boiled solid; no convulsions; dilatation of cervix. September 23, fetus delivered by craniotomy; patient very restless; one convulsion during night. Death 12.15 A.M., Sept. 24, 1925.

Postmortem Report: Very obese, weight 250 lbs.; edema of legs, hands and eyelids; heart, weight 410 gm.; liver, weight 1970 gm., a few hemorrhagic necroses; no endometritis.

The kidneys weighed 175 and 150 gm. respectively. The external surfaces were smooth, the cortices very cloudy.

Microscopically erythrocytes are found in many tubules, having

escaped from deeply congested glomeruli. In general there is only a slight increase of endothelial nuclei, but a few tufts are occluded by endothelial cells. There is a moderate thickening of the capillary basement membrane in most of the glomeruli, but it is much less pronounced than in Case 14 (Fig. 5).

CASE 5. (26-145) Gravidia I. 18 years of age. Admitted Feb. 12, 1926, about six and a half months pregnant. Had morning sickness during the early months of pregnancy. About one week before admission she developed vomiting and headache. Vomited eight or nine times a day for the past five days. Had a slight convulsion at 8 P.M., February 11. Two hours after entrance she began having convulsions which soon became almost continuous. February 12, blood pressure 148/98; pulse 108; temperature 99.4° F.; edema of eyelids. Urine: specific gravity 1018, albumin + + + +, sugar + +, many casts, erythrocytes and leucocytes, no acetone or diacetic acid. Blood: hemoglobin 80 per cent, erythrocytes 4,560,000, leucocytes 24,000, 95 per cent polymorphonuclears. Blood urea nitrogen 24.3 mg.; creatinin 1.87 mg. February 13, induced labor with delivery of a premature dead fetus. Coma. Death 6.20 P.M.

Postmortem Report: Slight edema of the legs; no jaundice; no excess of fluid in the serous cavities; heart, weight 260 gm.; liver, cloudy, no gross areas of necrosis; multiple thromboses of the dural sinuses; purulent ethmoiditis.

The kidneys weighed 120 and 125 gm. respectively. There was a slight dilatation of both pelves and both ureters. The external surfaces were smooth, the cortices cloudy.

Microscopically there is a slight enlargement of the glomeruli with a moderate narrowing of the capillaries, which is caused mainly by an increase of endothelial cells. There is only a slight thickening of the capillary basement membrane. The glomerular structure is similar to that shown in Fig. 6. The associated infection may be responsible for the endothelial proliferation.

CASE 6. (26-283) Gravidia I. 17 years of age. First seen by a physician at 8 A.M., March 22, 1926, in the first stage of labor. Her blood pressure was 150/110. She was delivered of a normal baby at 1.15 P.M. the same day and appeared normal at 3 P.M. At 4 P.M. she developed attacks of muscular twitching with cyanosis and retraction of the head, and passed into coma. At 5 P.M. the blood pressure was 180 systolic. 4 ounces of urine removed by catheter showed a heavy deposit of albumin. At 9 P.M. 6 ounces of urine were removed by catheter. Death, which occurred at 3 A.M. March 23, was preceded by a typical convulsion.

Postmortem Report: No edema; 200 cc. of clear fluid in the peritoneal cavity; heart, weight 300 gm.; liver, weight 1300 gm., multiple small hemorrhagic necroses.

The kidneys weighed 150 gm. each. There was moderate dilatation of the right pelvis and ureter. The external surfaces were smooth, the cortices cloudy.

Microscopically there is moderate enlargement of the glomeruli with narrowing and occlusion of the capillaries, caused chiefly by increase of the endothelial cells, but partly by thickening of the capillary basement membrane.

CASE 7. (26-725) Gravida I. 37 years of age. Admitted Aug. 4, 1926, in coma, about six and a half months pregnant. Said to have been well until early in the morning of August 4. She had three or four convulsions before admission. Blood pressure 195/130. Urine: specific gravity 1016, albumin +++++, a few hyaline and granular casts, no sugar, a few red cells and many leucocytes. Leucocytes 22,900. Blood urea nitrogen 22.9 mg. Blood sugar 0.30 per cent. A bag was inserted to induce delivery, but the patient died undelivered within three hours after admission without regaining consciousness.

Postmortem Report: No edema; slight icterus; 100 cc. of thin blood-tinged ascitic fluid; heart, weight 430 gm.; liver, weight 1910 gm., fatty, many large hemorrhagic necroses; uterus contained a fetus weighing 840 gm.

The kidneys weighed 190 and 185 gm. respectively. The external surfaces were smooth, the cortices pale with a yellowish tint.

Microscopically the glomeruli are enlarged and anemic. There is a very marked narrowing of the capillaries produced by thickening of the capillary basement membrane.

CASE 8. (26-965) Multipara. 40 years of age. Under observation during the latter part of her pregnancy. Her blood pressure was elevated, as high as 150 mm. Hg. at one time, and there was albumin in the urine. Labor started suddenly on Nov. 5, 1926, and the baby was born on the way to the hospital. The placenta was delivered in the hospital shortly after admission, at about 4 P.M. At 7 P.M., November 5, she began to complain of abdominal pain and backache. About 9 P.M. she vomited undigested food and was very restless and uncomfortable. At 11 P.M. she complained of severe pain in the chest and dyspnea. At 11:30 P.M. muscular twitchings and rigidity of the muscles of the face were noted. There were four such attacks, each of which lasted about thirty seconds. These attacks were followed by severe convulsions and coma. Systolic blood pressure at this time was 220 mm. Hg. At 6 A.M. November 6, she seemed conscious at intervals. Systolic blood pressure 135 mm. Hg. Ten ounces of urine removed by catheter at noon, November 6, showed albumin +++, many casts and erythrocytes. About 2 P.M. November 6, she went into coma again. Systolic blood pressure 200 mm. Hg. Death in coma 7:30 A.M., Nov. 7, 1926.

Postmortem Report: No edema; no jaundice; no excess of fluid in

the serous cavities except the pericardial, which contained 150 cc.; heart not enlarged; liver, extensive subcapsular hemorrhages.

The kidneys showed smooth external surfaces and cloudy cortices.

Microscopically the glomeruli are enlarged. There is a marked narrowing of the glomerular capillaries produced chiefly by thickening of the capillary basement membrane. There is a slight increase of endothelial cells.

CASE 9. (28-383) Gravidia I. 18 years of age. Admitted March 11, 1928, near full term. No symptoms until March 10, 1928, when she complained of epigastric pain. On this date her systolic blood pressure was 170 mm. Hg., and the urine showed a large amount of albumin. On March 11, labor pains began. On March 12, noon, blood pressure 188/120; heavy albuminuria and casts. Labor began in the early afternoon. One convulsion at 6 P.M., and another at 8 P.M. Forceps delivery of dead infant. Death at 10:15 P.M., thirty minutes after completion of labor.

Postmortem Report: Moderate edema of legs and labia; no fluid in serous cavities; heart, weight 300 gm.; liver, weight 2150 gm., numerous small hemorrhagic necroses.

Each kidney weighed 150 gm. The external surfaces were smooth, the cortices soft and cloudy.

Microscopically the glomeruli are enlarged and anemic. The narrowing of the capillaries is due to a marked thickening of the capillary basement membrane.

CASE 10. (28-526) Gravidia I. 38 years of age. Admitted April 12, 1928. Well until April 12, 4 A.M., when she suddenly developed a convulsion. No examination before this date. A catheterized specimen of urine boiled solid. Nine convulsions before admission. In coma when admitted. Pregnancy estimated at seventh month, slight edema, cervix moderately dilated. Blood pressure 200/80; one convulsion shortly after entrance to hospital. Blood pressure fell to 125/80. Urine: large amount of albumin and many casts. Hemoglobin 85 per cent; erythrocytes 4,200,000; leucocytes 16,800. Blood urea nitrogen 17.7 mg. On April 13, blood pressure could not be obtained, but after stimulation it rose to 230/110. Vorhees bag inserted. Dead fetus delivered spontaneously at 9 P.M. Partial recovery of consciousness. April 14, marked oliguria, rapid fall of blood pressure. Death April 14, 1926.

Postmortem Report: Achondrodysplasia; no edema; 750 cc. of ascitic fluid; heart, weight 220 gm.; terminal bronchopneumonia; liver, weight 1725 gm., fatty, multiple hemorrhagic necroses.

The kidneys weighed 140 gm. each. The external surfaces were smooth, the cortices cloudy.

Microscopically the glomeruli show narrow capillaries and very little blood. The narrowing is due chiefly to thickening of the capil-

lary basement membrane, but there is some increase of endothelial cells.

CASE 11. (28-533) Gravida I. 25 years of age. Always in excellent health. Confinement expected April 20, 1928. Under constant observation during pregnancy, and nothing abnormal found until April 12, when routine urine examination revealed a moderate albuminuria. On this date no casts were found, and the systolic blood pressure was 120 mm. Hg. The patient was put to bed on a restricted diet. April 14, heavy albuminuria; puffiness about the eyes. On April 15, a severe headache developed and she was taken to a hospital. The first convulsion developed shortly after admission. Blood pressure 160 mm. Hg. systolic. There were five more convulsions within the next twenty-four hours, in spite of large doses of sedatives. Caesarian section was performed at 10:30 A.M., April 16. Living child. Mother never regained consciousness. Death 6:20 A.M., April 17, 1928.

Postmortem Report: No edema; 500 cc. of blood-tinged peritoneal fluid; 1000 cc. of clear fluid in each pleural cavity and 500 cc. in the pericardial cavity; marked edema of the lungs; distention of the stomach; heart, weight 350 gm.; no hemorrhages or necroses in the liver, cloudy swelling.

The kidneys weighed 200 gm. and 175 gm. respectively. The external surfaces were smooth. On section the cortices were cloudy and pale.

Microscopically, the glomeruli are slightly enlarged and anemic. There is a marked increase of endothelial nuclei in the glomerular capillaries which is the chief cause of the narrowing (Fig. 6). However, in some loops the thickening of the basement membrane is the main cause of the reduced caliber of the capillaries.

This lesion bears a striking resemblance to acute glomerulonephritis, but there are very few intracapillary fibers, no leucocytes, and no epithelial crescents.

CASE 12. (28-1228) Gravida VII. 39 years of age. According to her husband's statement, she had been fairly well during this pregnancy, except for occasional headaches. When he left home in the morning she said that she did not feel well. When he returned from work that evening he found her unconscious. A little later there was a precipitate spontaneous delivery of a dead infant. About eighteen hours after labor she was brought to the hospital. Upon admission she was in deep coma. Death occurred about ten minutes after admission.

Postmortem Report: Slight edema of legs; no jaundice; 500 cc. of clear fluid in the right pleural cavity; heart, weight 420 gm.; edema of right lung; liver, weight 2950 gm., fatty, numerous hemorrhagic necroses on section; no endometritis.

Kidneys weighed 200 and 205 gm. respectively. The external surfaces were smooth, the cortices cloudy.

Microscopically the glomeruli are moderately enlarged, and their capillaries are markedly narrowed. There is a marked thickening of the capillary basement membrane and some increase of endothelial cells. The changes are due chiefly to thickening of the basement membrane.

CASE 13. (30-318) 28 years of age. Confinement expected March 22, 1930. She had been under the constant care of a physician since Sept. 6, 1929. Numerous readings showed a range of the systolic blood pressure from 124 to 138 mm. Hg. The last reading, on February 21, was 138/80. Many examinations of the urine had been made, and occasionally a trace of albumin was found, but there was no albumin at the last test on February 21. She was visiting with friends on the evening of February 27, and was apparently well, but was found dead on Feb. 28, 1930.

Postmortem Report: No edema; no jaundice; the tongue was protruding and caught between the teeth; no excess of fluid in the serous cavities; heart, weight 355 gm.; liver, weight 1950 gm., fatty, numerous hemorrhagic necroses; 44 cm. fetus in the uterus; postmortem urine specimen showed abundant albumin.

The kidneys weighed 145 and 120 gm. respectively. The external surfaces were smooth, the cortices cloudy.

Microscopically, the glomeruli are slightly enlarged and the glomerular capillaries are very small. The narrowing of the capillaries is caused by a marked thickening of the capillary basement membrane.

CASE 14.* (31-476) Gravida I. 18 years of age. When first examined, June 21, the patient was at full term and labor had begun. There was moderate edema of the lower extremities, and the blood pressure was 150/100. No headache, nausea, or other toxic symptoms. The urine showed albumin ++++. She was put to bed under treatment. At 4:30 P.M., June 21, she had a convulsion. Sedatives were administered. At 6 A.M., June 22, a second convulsion occurred. She became semiconscious, blood pressure 150/95. She had another convulsion during the forenoon. At 3 P.M. the cervix was fully dilated. A full term stillborn infant was delivered with low forceps. June 23, somewhat stuporous; deepening jaundice; edema slightly more pronounced; pulse 100; temperature 99 to 100° F. Glucose was given intravenously as on the previous day. Alkaline reserve 53. Hemoglobin 60 per cent. The urine, by catheter, showed albumin ++++, sugar, a few red cells, no casts, no acetone. Blood pressure 148/98. The total fluid intake from 7 A.M. June 23 to 7 A.M. June 24

* This case was made available through the courtesy of Dr. Warren C. Hunter, of Portland, Oregon.

was 2220 cc., and the total output of urine during this twenty-four hour period was 295 cc.

June 24, the patient seemed improved. There was very little stupor. Edema and jaundice unchanged. Blood pressure 148/98 to 152/64. Therapy: glucose, sodium bicarbonate, diuretin. Fluid intake 4680 cc.; urine 330 cc.; albumin++.

June 25, fluid intake 7100 cc.; urine 220 cc. Blood pressure 148/60. Temperature 101.6° F.; pulse 120.

June 26. Therapy: glucose; alkali discontinued since alkali reserve had risen to 140. Fluid intake 4600 cc.; urine 100 cc.

June 27. One hour after administration of glucose the blood sugar was 413 mg., and the alkali reserve 147. Insulin was given. Temperature 103.8° F.; pulse 120. Fluid intake 1740 cc.; urine 66 cc. The patient gradually became weaker and died at 4 A.M., June 28.

Postmortem Report: Severe jaundice; slight pitting edema of the ankles; ascites, 3000 cc.; marked bilateral hydrothorax; atelectasis of lungs; heart, weight 280 gm., no gross disease; liver, weight 2010 gm., numerous areas of hemorrhagic and anemic necrosis, fatty; cystitis; no endometritis.

The combined weight of the kidneys was 480 gm. The external surfaces were smooth. On section the cortices were pale and moist.

Microscopically the glomeruli are all moderately enlarged and anemic. There is a striking increase in the thickness of the capillary basement membrane (Fig. 5). The endothelial nuclei are not definitely increased in number. No fat is demonstrable in sections stained with Sudan III.

Anatomical Changes in Eclampsia

The Liver: A comprehensive review of the changes in the liver in eclampsia may be found in Fahr's article in Hinselmann's monograph. Only a brief summary need be given here, since this paper deals primarily with the renal lesions only. Degenerative lesions of some type are found in the liver almost constantly. The characteristic gross lesions are irregular areas of hemorrhage associated with necrosis or atrophy of the liver cords (hemorrhagic necrosis) and small areas of anemic necrosis. These macroscopic lesions are found only in eclampsia, and a diagnosis of eclampsia may be made on their presence, even in the absence of clinical signs of eclampsia. However, macroscopic necrotic lesions are not always present. Many writers report them absent in a notable percentage. In 2 of our 14 cases, no gross necroses were found. However, when a thorough microscopic examination is made, as has been emphasized by Lubarsch, Schmorl, and Fahr, necrosis and degeneration of the liver

cords are seldom entirely absent. Cloudy swelling and fatty degeneration of the hepatic cords are also found frequently. Fibrin thrombi in the small branches of the portal vein and areas of capillary ectasia with atrophy of the enclosed hepatic cords are commonly present. Thrombosis of small vessels seems sufficient to account for the areas of necrosis and hemorrhage, but the diffuse injury of the hepatic cells is best explained as the effect of a circulating toxic substance.

The Kidneys: The kidneys are affected in practically all cases of eclampsia. There are occasional reports in the literature in which the kidneys are described as normal. Seven out of 368 cases reviewed by Prutz, 1897, were considered normal. Fahr is inclined to doubt reports of normal kidneys in eclampsia, and it is evident that no such report should be accepted unless a careful macroscopic and microscopic examination has been made. However, in view of the rare instances of eclampsia without albuminuria, it seems possible that the kidneys may escape injury in exceptional instances.

The macroscopic changes are constant and easily recognized. Aside from the rare cortical necroses, which will be discussed separately, the only change is cloudy swelling. The kidneys are usually slightly enlarged and their external surfaces are smooth. On section the cortices are pale and cloudy and occasionally a yellowish tinge is noted. There is some variation in the intensity of the cloudy swelling in different instances. The macroscopic changes are therefore not pathognomonic of eclampsia, the same lesion being found in a large number of toxic and infectious processes.

On microscopic examination the tubules show the changes characteristic of cloudy swelling. The cells of the secreting tubules are usually somewhat swollen and often they contain small fat droplets. In the lumina of the tubules, casts and precipitated albumin are usually observed. Fahr emphasizes the importance of hemoglobin in the casts. In the more severe injuries there may be some evidence of necrosis and degeneration of some of the tubular epithelium. The tubular lesion is likewise not peculiar to eclampsia. It is the typical effect of toxins or toxic substances in the circulating blood, but it may also result from anemia caused by spasm of the renal arteries.

The Glomerular Lesions: Pels-Leusden, 1895, recognized clearly that the glomeruli are especially involved in eclampsia. It is true

that he regarded the lesion as acute glomerulonephritis, but he noted that the glomeruli are enlarged and pale and contain only a little blood.

Schmorl, 1902, described hyaline thromboses in the glomerular capillaries. Fahr pointed out later that this lesion occurs only infrequently. It was not found in any of our cases. Schmorl disagreed with the prevalent view at that time that the eclamptic kidney indicates glomerulonephritis.

Löhlein, 1918, gave the first concise and accurate description of the characteristic glomerular lesion in eclampsia. He noted thickening of the walls of the glomerular capillaries, decrease of erythrocytes, swelling of the glomerular epithelium and a slight increase of intracapillary cells. He stated that the lesion suggests glomerulonephritis but is different. Löhlein did not accept "nephrosis" as an entity, and it is not clear whether he regarded this glomerular change as degenerative or inflammatory.

Fahr, 1920 and 1924, has given accurate descriptions and illustrations of the glomeruli in eclampsia. He described thickening of the capillary walls with clumping and fusion of the loops, and eventually hyalinization of some of the tufts. No increase of glomerular nuclei was found as a rule, but there were some exceptions. Fahr considers this glomerular lesion peculiar to eclampsia, and calls it a glomerulonephrosis. He interprets it as a degenerative change and believes that it is best explained by the toxic theory of eclampsia.

Pohl, 1927, noted the enlargement of the glomeruli, but did not comment upon their structure.

My own observations are in almost full accord with those of Löhlein and Fahr, but I have succeeded in showing the structural changes in the glomeruli in greater detail by means of a special stain. In sections stained with hematoxylin and eosin, the glomeruli attract attention by reason of their slightly hyaline appearance and their small empty capillaries (Fig. 1). The increase in size is usually only moderate, but sometimes quite pronounced. As a rule there is no increase in the number of nuclei, but in some kidneys a definite increase is observed. Under high magnification a marked thickening of the walls of the capillaries is readily seen, as has been described by Löhlein and Fahr. No capillary thrombi were found in any of my cases.

The detailed structure of the glomerulus is brought out in a re-

markable way by the azo-carmin stain. The technique of this stain has been published by McGregor, and the reader is referred to her articles for its application to the normal glomerulus and glomerulonephritis. The great advantage of this stain is that it demonstrates the capillary basement membrane sharply and affords an easy distinction between endothelial and epithelial cells. The endothelial cells lie on the inner surface of the membrane, and the glomerular epithelial cells on its outer surface.

When the azo-carmin stain is applied to the glomerulus in eclampsia, it is easily seen that the thickening of the capillary wall is due, almost entirely, to a massive thickening of the capillary basement membrane (Fig. 5). The glomerular epithelial cells are only slightly altered. Occasionally they contain fine droplets of fat or hyaline granules, but they show no evidence of proliferation. As a rule the endothelial nuclei are slightly increased in number. The narrowing of the lumina of the capillaries is evidently due, usually, to thickening of the capillary basement membrane. The capillaries are rarely completely obstructed, but in some tufts their thickened walls are in contact and they seem to be totally occluded. Capillaries of this type appear hyaline in hematoxylin-eosin preparations.

The degree of thickening of the capillary basement membrane is fairly uniform in all the capillaries of all the glomeruli in each individual case, but it varies in different cases. In the 17 cases which showed thickening of the basement membrane, it was moderately thickened in 2 and markedly so in 15.

The thickened basement membrane is not a homogeneous structure. It is composed of parallel layers (Fig. 5). The thickening cannot be interpreted as a simple swelling (edema). It must represent an increase of substance in the membrane. There is no anatomical explanation of its increased permeability.

In 3 cases the increase of endothelial nuclei is striking (Fig. 6), and a definite but moderate increase was noted in 2 others. The endothelial nuclei lie on the inner surface of the basement membrane, and are easily distinguished from the epithelial cells. Where the endothelial nuclei are notably increased, the thickening of the basement membrane is correspondingly less pronounced.

Fahr thinks it possible that the endothelial increase is due to a complicating infectious process, and not to eclampsia *per se*. How-

ever, if a toxic substance is concerned in eclampsia there is no sound reason for believing that it cannot cause an increase of glomerular endothelium. Hyperplasia of the glomerular endothelium is by no means limited to clinical glomerulonephritis, but is found in varying degree in a wide variety of infectious processes.

There are, however, striking differences between the eclamptic glomerulus and clinical acute glomerulonephritis, even when the former shows a notable increase of endothelial nuclei. The glomeruli in eclampsia are smaller, the basement membrane is much thicker, there are no polymorphonuclear leucocytes, no intracapillary fibers and no epithelial crescents. In glomerulonephritis there is much more cytoplasm about the endothelial nuclei. The two types of glomeruli may be distinguished without difficulty.

Fahr mentions small patches of hyaline degeneration in the afferent glomerular arterioles as an occasional finding. In 2 of my cases a slight arteriolar sclerosis was found.

Symmetrical Necrosis of the Cortex of the Kidneys: The first case of this kind was described by Bradford and Lawrence in 1898, and since that time 17 similar cases have been reported: Schuppel, 1904; Lloyd, 1906; Griffith and Herringham, 1906; Klotz, 1908; Jardine and Teacher, 1911, 2 cases; Torrens, 1911; Jardine and Kennedy, 1913, 3 cases; Herzog, 1913; Rolleston, 1913; Glynn and Briggs, 1914-1915; Jardine and Kennedy, 1920; Cruickshank, 1923; Geipel, 1925; and Carson and Rockwood, 1926. Poten's case, 1906, probably belongs to this group.

The outstanding clinical features are præclamptic symptoms with or without convulsions, followed by severe oliguria or complete anuria, and ending in death within a few days. Ten of the reported cases were in multiparae, and 6 in primiparae. Age seems to be of no significance. In 8 cases no convulsions are mentioned, an unusually large proportion of "eclampsia without convulsions." However, definite præclamptic symptoms are described in every instance. The stage of gestation varies from three and a half months to full term. Typical uremic symptoms are commonly absent. The scanty urine contains blood, albumin and casts.

Macroscopic necrosis of the liver was noted in 3 cases. In 6 cases it is clearly stated that no macroscopic necrosis was present, but in 3 of these the liver was fatty. The frequent absence of convulsions and typical necrosis of the liver raises a doubt as to whether this

group should be classified as eclampsia. They are truly not all typical eclampsia, but they may at least be grouped with pre-eclampsia on the basis of the clinical symptoms.

The kidneys in every case are similar. There is an almost complete necrosis of the cortices of both kidneys. There is thrombosis of the interlobular arteries and usually of the vasa afferentia also. Sometimes the thrombosis extends into the glomerular capillaries. The large branches of the renal arteries are free from thrombi. The prevailing opinion is that the thrombosis is primary and that the necrosis results from infarction. This interpretation is strongly supported by the finding of a thin layer of living cortex immediately under the capsule which is supplied by anastomoses with capsular arteries. Preëxisting disease of the renal arteries is described in some cases, but this is inconstant and apparently unrelated to the thrombosis. The cause of the thrombosis is not known, but it is best explained as a result of a toxic substance or a coagulant in the circulating blood.

2. ECLAMPSIA WITHOUT CONVULSIONS

This group includes fatal toxemias, usually with some preëclamptic symptoms, but without convulsions. The diagnosis is established by the finding of typical necrosis of the liver at postmortem.

Ordinarily there is not much justification for this subgroup of eclampsia. Cases are frequently reported in which only one convulsion occurred (see Case 4); and others are reported where muscular twitchings but not true convulsions were present. The following protocol records a case which differs clinically in no way from typical eclampsia, except in the absence of convulsions.

CASE 15. (30-1395) Para II. Negress, 37 years of age. Admitted Sept. 15, 1930, about seven months pregnant. Moderate nausea and vomiting during first three months. No symptoms after the third month until the day of admission, when she began to vomit. No visual disturbance. About six weeks before admission a physician took her blood pressure and told her it was high. September 15, blood pressure 266/180; slight edema of lower extremities. September 16, coma; blood pressure 170/80. Urine: large amount of albumin, large number of erythrocytes (gross hematuria), specific gravity 1020. Blood urea nitrogen 33 mg.; creatinin 3.3 mg. The eyegrounds showed some old and some recent hemorrhages, and some edema of the disc. Temperature, 104° F. No convulsions. Death Sept. 16, 1930.

Postmortem Report: Moderate edema of the lower extremities; no jaundice; a little excess of fluid in the serous cavities; heart,

weight 306 gm.; bilateral bronchopneumonia; liver, weight 1384 gm., fatty, many areas of hemorrhagic necrosis; uterus contained a 37 cm. fetus.

The kidneys weighed 180 and 164 gm. respectively. The external surfaces were smooth. The cortices were pale with a yellowish tinge.

Microscopically the glomeruli are enlarged and anemic. Small patches of hyalin are seen in the arterioles and glomeruli. There is a very marked narrowing of the glomerular capillaries produced by thickening of the capillary basement membrane. There is a slight increase of endothelial cells.

It may be contended that this is an instance of pregnancy in a woman with primary hypertension, since there is a slight renal arteriolar sclerosis, but the presence of hemorrhagic necroses in the liver establishes the disease as eclampsia. Primary hypertension as a complicating influence cannot be excluded.

The following case is, in all probability, eclampsia, although convulsions and necrosis of the liver are both absent.

CASE 16. (30-1760) Gravida I. Last menstrual period March 1, 1930. May, 1930, a trace of albumin was found in the urine. August, 1930, trace of albumin in the urine, no other signs of toxemia; blood pressure 118/70. In the next two months there was a steady increase of albumin. November 18, albumin + + +, a few casts and erythrocytes; blood pressure 170/108. November 25 labor was induced, but the patient died undelivered. There were no convulsions.

Postmortem Report: Edema of one leg; no excess fluid in the serous cavities; heart, weight 350 gm., acute rheumatic mitral endocarditis; liver, weight 2665 gm., cloudy swelling, no necroses; twin pregnancy, each fetus 47 cm. long.

The kidneys weighed together 400 gm. The external surfaces were smooth. The cortices were pale and cloudy on section.

Microscopically the glomeruli are definitely enlarged, and they contain practically no blood. There are no epithelial crescents. The greater number of glomeruli show only a little endothelial increase, but in a great many the increase of endothelial cells is very prominent. There is a striking thickening of the capillary basement membrane in all the glomeruli, such as is shown in Fig. 5, a lesion which is characteristic of eclampsia and not of glomerulonephritis. A few glomeruli show intracapillary fibers.

In view of the infection which was present, namely, rheumatic endocarditis, the endothelial increase and the intracapillary fibers may be interpreted as a result of infection superimposed on an eclamptic kidney. It cannot be considered as acute glomerulonephritis complicating pregnancy, since the lesions are chiefly of the eclamptic type.

The two preceding cases are easily recognized clinically as belonging to the eclamptic group, but much more atypical cases are on record.

Schmorl, 1902, called attention to a type of toxemia which differs sharply clinically from typical eclampsia, and is only recognized with certainty as eclampsia by the finding of necrosis of the liver at postmortem. It is convenient to designate this group as "eclampsia without convulsions" or "atypical eclampsia." Over 40 cases of this type have been reported. The literature on this topic will not be reviewed here. The reader is referred to the papers of Schmorl, Schmid, Ranzel, Liebmann, Pohl, Bock, and Wronski.

The attack may develop before, during, or after labor. Usually there are some warning preëclamptic symptoms, but sometimes the patient sinks into coma or circulatory collapse without any previous signs of toxemia. In the complete absence of preëclamptic symptoms, the diagnosis can hardly be established ante mortem. Albumin may be absent until shortly before death. The blood pressure tends to fall.

The disease is recognized as eclampsia by the finding of the typical necroses of the liver. In some of the most atypical cases there is also a large intracranial hemorrhage.

The kidneys in atypical eclampsia have not been studied in detail microscopically. The reports indicate that the same macroscopic changes are present as in typical eclampsia.

It is to be noted that bilateral symmetrical necrosis of the cortex of the kidneys gives rise to an atypical clinical form of eclampsia.

3. PREËCLAMPSIA

Preëclampsia is characterized by the presence of the symptoms and signs which usually precede the eclamptic convulsion. The most important of these are hypertension, albuminuria, visual disturbances, edema, headache, nausea and vomiting, vertigo, and so

on. The individual symptoms and signs vary greatly in their prominence in different cases. When severe visual disturbances are present there is great danger that convulsions will develop. Moderate edema without other preëclamptic symptoms is not of serious significance, and is probably not a true preëclamptic sign.

Preëclampsia recurs oftener in subsequent pregnancies than typical eclampsia, and recovery from severe preëclampsia is apparently more prolonged.

The clinical phenomena indicate that the same type of lesion is present in preëclampsia as in typical eclampsia, although it is presumably less severe. Heynemann described a case of preëclampsia in which death was due to premature separation of the placenta. The characteristic changes were present in the liver and kidneys. Even the typical glomerular lesions were found. Heynemann states that 6 similar cases have been reported. This would seem to establish the essential identity of eclampsia and preëclampsia. Cases 15 and 16 might well be classified as preëclampsia rather than as eclampsia without convulsions.

Changes in the Kidneys Subsequent to an Attack of Eclampsia

What permanent damage, if any, does eclampsia produce in the kidneys? Is the acute glomerular lesion reversible or does it lead to partial or complete obliteration of glomeruli with atrophy of their corresponding tubules? Does a clinical chronic renal disease result from eclampsia; and, if so, what are its characteristic features? These are some of the questions to which we shall now direct our inquiry.

A number of clinical reports deal with the question of chronic renal disease following eclampsia. Leyden, 1886, reports one case of chronic nephritis following eclampsia. The woman was said to have been well before pregnancy, but apparently no study was made with respect to the presence of latent renal disease. The postmortem revealed contracted kidneys.

Koblanck, 1894, reëxamined 77 women who had had eclampsia (time of recheck not given): 59.7 per cent had no albumin; 16.9 per cent had a transitory trace of albumin; 15.4 per cent had catarrh of the urinary tract; and 6.5 per cent (5 cases) had nephritis. No details of the nephritis were given.

Meyer-Wirz, 1904, observed 35 postmortems in instances of eclampsia, and found 3 with chronic indurated kidneys. He also found that the great majority of eclamptics were free from albumin on dismissal from the hospital, but he mentioned 9 cases in which albumin was still present.

Zangemeister, 1913, found that a chronic nephritis remained in 7 per cent of eclamptics. He thought that the majority of these developed from the eclamptic kidney and not from a previous chronic nephritis.

Baisch, 1913, traced 110 women who had had an attack of eclampsia (60 cases) or severe præclamptic symptoms (50 cases). Of these 110 patients, 9 were dead, and 11 were permanent invalids. Only 40 per cent were entirely well. No information is given as to the cause of death or invalidism. No evidence of renal disease was presented.

Wolff and Zade, 1914, found 2 out of 23 eclamptics, whom they re-examined some years later, with evidence of chronic nephritis. The first patient had no albumin on dismissal, and when reëxamined had hypertension without albumin. The second had no albumin on dismissal, and on reëxamination had hypertension, 200/120, and a trace of albumin. Two of seven præclamptics had similar evidence of a chronic nephritis on reëxamination. These cases can hardly be accepted as proof that a chronic nephritis may develop from the eclamptic kidney, but such an interpretation is possible. They may represent instances of primary hypertension developing independently of eclampsia.

Sachs, 1918, found 81 of 87 eclamptics entirely well on reëxamination some years later. Four were dead (one from a recurrent eclampsia, three from intercurrent disease). Two showed signs of chronic nephritis.

Hüssy, 1921, doubts the transition of the eclamptic kidney into chronic nephritis. He did not see any instance of this in his experience.

Breuning, 1924, in a report of 88 cases of eclampsia, states that 88 per cent of those who survived were free from albumin when dismissed from the hospital.

Heynemann, 1924, gives a full account of the results of reëxamination of 45 patients who had eclampsia and 7 who had pronounced præclamptic symptoms. His paper should be consulted for the

detailed findings in each case. He concludes that not infrequently there is evidence of renal injury not only on dismissal but on later re-examination. This is indicated by the presence of albuminuria or hypertension, or both. He interprets these findings in most instances as evidence of delayed healing, but thinks that the cases with hypertension only may be due to disease of arterioles and capillaries resulting from eclampsia. He finds no convincing evidence in his experience that chronic renal disease results from eclampsia. Patients with pronounced preëclamptic symptoms show slower healing than those with eclampsia.

Fahr, 1924, in the light of his pathological studies, expresses the opinion that it is possible for the eclamptic kidney to give rise to chronic renal disease.

Zondek and Jacobowitz, 1924, reëxamined 38 patients who had had eclampsia or preëclampsia, one to seven years later. There was one case of chronic nephritis which they believe was present before the attack of eclampsia. In a few cases there was evidence of a slight renal disturbance. They conclude that it is possible that eclampsia gives rise to chronic nephritis, but it must be very rare.

Döderlein, 1925, reëxamined 26 patients eight months to fifteen years after eclampsia and 16 were entirely normal. Slight albuminuria with normal kidney function was present in 3 patients and 7 showed albumin and casts, with some evidence of decreased renal function, which he interprets as indicating a chronic nephrosis resulting from eclampsia.

Bund, 1925, partly by correspondence, followed 39 patients who had eclampsia. Four had chronic nephritis which he attributed to eclampsia.

Nevermann, 1927, reëxamined 60 patients, one to twenty-three years after eclampsia. Thirty-seven were studied more than ten years after the attack. Twenty-seven were entirely normal. The others had various complaints such as headache, poor memory, visual disturbances and edema of the legs. Eight women had hypertension (systolic pressure 140 to 170 mm. Hg.). Three had albumin and casts: (a) one year after eclampsia, trace of albumin, blood pressure 118 mm. Hg.; (b) eclampsia in 1903 and again in 1905, albuminuria on dismissal each time, since 1905 seven abortions and premature labors; in 1925, blood pressure 170 mm. Hg., large amount of albumin; (c) eclampsia in 1921, mild preëclampsia with abortion in

1925 (albumin), normal in 1926. There was only one patient with a persistent renal lesion. Nevermann believes that chronic nephritis rarely, if ever, develops from eclampsia.

Peckham, 1929, found 17 (23 per cent) of 77 women with chronic nephritis on reëxamination one year after eclampsia. Nephritis developed somewhat oftener in those who were albumin-free at the end of three weeks than in those who had albuminuria at that time. Peckham does not explain the criteria on which his diagnosis of chronic nephritis is based.

Schmechel, 1929, in a large experience with eclampsia, knew of only one patient who developed chronic renal disease. He thought that this patient probably had renal disease before pregnancy.

Kobes, 1930, found that 19 of 32 eclamptics had albuminuria at the end of the third week after the attack. Reëxamination of these 32 women from three to eighty-five months later showed only 3 with albuminuria (four months, seven months, ten months later).

Fourteen of 19 preëclamptics had abnormal urine when reëxamined, but 13 of these had a history of renal disease prior to pregnancy. Two women had evidence of contracted kidney. Kobes was unable to decide whether or not eclampsia causes chronic renal disease.

Seitz, 1930, found 78 eclamptics all entirely well at the end of eight weeks. The preëclamptics (27 in number) showed a little slower healing. A few were not normal after fourteen weeks.

It is difficult to draw definite conclusions from the literature, since the experience of different observers has not been uniform. However, it appears that evidence of mild renal injury is frequently found a long time after an attack of eclampsia. Whether or not the cases of definite chronic nephritis that follow eclampsia are the outcome of the eclamptic kidney cannot be decided. In order to establish such an origin for the nephritis, it must be shown by careful examination that nephritis was not present prior to the pregnancy. None of the reported cases of contracted kidneys were studied before pregnancy.

The clinical evidence indicates that the eclamptic kidney usually heals rapidly, but in some instances very slowly. There is a possibility that it may rarely pass into a definite chronic nephritis with contracted kidneys, but there is no convincing evidence that this occurs.

We may next inquire if there is any anatomical evidence of a permanent renal lesion resulting from the eclamptic kidney. Microscopic examination of kidneys from women who had eclampsia a long time previously should reveal permanent lesions if any are present, but apparently no such studies have been made. It will be interesting, therefore, to study in detail the old lesions in Case 3, in which there is a history of a previous attack of eclampsia seven years before death. In the protocol (Case 3), the histological changes are fully described (see also Figs. 2, 3 and 4). It is clear that the acute lesions of eclampsia did not heal entirely in this instance. The capillary basement membrane thickened to the point of complete obliteration of groups of glomerular capillaries. Disuse atrophy of the tubule developed to a degree proportionate to the diminished capillary bed of its glomerulus.

The clinical evidence in this case is lacking, but there is strong anatomical evidence of chronic renal disease resulting from eclampsia. It is quite different from ordinary chronic glomerulonephritis in its pathogenesis, but the end result is obstruction of glomerular capillaries and tubular atrophy. It may be said, therefore, that chronic renal disease may result from the acute lesion of eclampsia, but it is a special type differing from the known forms of chronic renal disease in its structure and pathogenesis. On the basis of the anatomical structure, one would expect to find hypertension and renal insufficiency clinically.

4. HYPEREMESIS GRAVIDARUM

In general this form of toxemia is different from eclampsia, but cases occur which show some of the features of eclampsia, and raise a doubt whether these two forms of toxemia are entirely distinct entities.

The outstanding clinical feature of hyperemesis gravidarum is obstinate vomiting developing in early pregnancy, and the characteristic lesion found at postmortem is fatty degeneration of the liver. In some instances a severe anemia or a toxic myelitis dominates the clinical picture to such an extent that we seem to be dealing with a separate entity and not with hyperemesis gravidarum.

Two fairly typical cases of hyperemesis gravidarum are reported by Harbitz. The first case, a woman 25 years old, began to vomit in

the second month. The vomiting became very severe, and she became almost blind. Death occurred in the third month, forty-eight hours after the uterus was emptied. The liver and kidneys were fatty. There were no necroses in the liver. The second case, a woman 22 years old, vomited for three months and died in the fourth month. The same lesions were found at postmortem as in the first case.

The following four cases came under my observation:

CASE 17. (29-1213) Gravidia I. 39 years of age. Admitted Aug. 14, 1929. About four months pregnant. Last menses in April. Vomiting more or less continuously since the latter part of May. Complaints of vomiting, weakness and loss of weight. Blood pressure 100/70. No edema. Some mental disturbance. Slight icteric tint in the sclera. Moderate enlargement of the thyroid of two years' duration. Hemoglobin 85 per cent; erythrocytes 4,060,000; leucocytes 9,350. Differential count normal. Urine showed sugar, acetone and diacetic acid, no albumin. Blood urea nitrogen 14 mg. No improvement under treatment. Vaginal hysterotomy and craniotomy. August 21, collapse. Death Aug. 22, 1929.

Postmortem Report: Slight jaundice; no edema; no fluid in serous cavities; heart, weight 250 gm.; liver, weight 1150 gm., diffuse fatty metamorphosis; colloid goiter; no gross changes in pancreas.

Kidneys weighed 140 and 145 gm. respectively. Slight dilatation of right pelvis and ureter. No other gross changes.

Microscopically the glomeruli show a normal structure.

This is a typical case of hyperemesis gravidarum, showing none of the features of eclampsia, and not complicated with anemia or toxic myelitis. The kidneys were normal. The characteristic glomerular lesion of eclampsia is absent.

CASE 18. (26-238) Gravidia I. 20 years of age. About Dec. 25, 1925, she first developed nausea and vomiting. She became acutely ill Jan. 1, 1926, and has vomited almost continuously since that date whenever she attempted to eat. Admitted Feb. 2, 1926, three months pregnant at this time. The vomiting improved notably under treatment. The urine showed albumin, acetone and diacetic acid. Discharged February 26, much improved. Readmitted, March 2, complaining of a sore throat and disturbances of vision. Her vision was so impaired that she could barely recognize faces. Hemorrhages were visible in the retinae. Blood pressure 114/80. Temperature ranged between 100 and 103° F. Pulse very rapid. Leucocytes 7,000. Blood urea nitrogen normal. Urine showed albumin. Death March 9, 1926.

Postmortem Report: No edema; no jaundice; 200 cc. of blood-tinged ascitic fluid; heart, weight 220 gm., soft myocardium; liver, weight 1515 gm., very fatty, no hemorrhages or necroses; uterus contained a four months' fetus; no endometritis.

Each kidney weighed 150 gm. The external surfaces were smooth. The cortices were very pale, and two small abscesses were found in the cortex of one kidney. There were many petechial hemorrhages in the pelves. Microscopically the glomeruli are practically normal.

In the foregoing case death was apparently due to bacteremia rather than to toxemia of pregnancy. The retinal hemorrhages with loss of vision suggest eclampsia, since this is a rare complication of a simple septicemia. There was no hypertension. The extreme fatty degeneration of the liver is to be referred to toxemia of pregnancy. The glomeruli do not show the distinctive lesion of eclampsia.

CASE 19. (28-197) Gravida II. 24 years of age. Admitted Jan. 28, 1928. First pregnancy three years ago terminated in abortion at three and a half months. Duration of this pregnancy about seven and a half months. Vomiting began during the third month of pregnancy. She would vomit after every meal. The vomiting then stopped for three months, but recurred in the sixth month. For the past six weeks she has retained practically nothing that was taken by mouth. About two weeks before admission she developed difficulty in swallowing, which persisted. January 28, blood pressure 100/85. The urine on two examinations showed a faint trace of albumin, many hyaline casts, and a few erythrocytes. Hemoglobin 50 per cent. Erythrocytes 2,100,000. High color index. Blood smear showed moderate poikilocytosis and anisocytosis, no nucleated reds or polychromatophilia. Blood Wassermann negative. Blood urea nitrogen 17.8 mg. The vomiting was not relieved by treatment. On February 2, she suddenly went into labor and a living child was born spontaneously. About six hours after labor she became dyspneic. There were no convulsions. Death 11:35 A.M., Feb. 3, 1928.

Postmortem Report: No edema; 100 cc. of clear fluid in the pericardial cavity, none in the other serous cavities; heart, weight 198 gm.; liver, weight 1445 gm., light brown color, no areas of necrosis, very little fat.

The left kidney weighed 125 gm., the right 110 gm. Submucosal hemorrhages in the left pelvis. The external surfaces were smooth, the cortices pale.

Microscopically the glomeruli are moderately enlarged and anemic. There is a definite narrowing of the glomerular capillaries, produced by a marked thickening of the capillary basement membrane.

On clinical grounds this case may be interpreted as hyperemesis gravidarum complicated by severe anemia. The typical liver lesion of hyperemesis is, however, not present. There was only a faint trace of albumin in the urine, and there was no hypertension, but the characteristic glomerular lesion of eclampsia is present.

CASE 20. (30-3) Gravida VI. 30 years of age. Admitted Dec. 30, 1929. She was about four months pregnant, had been ill for two months, and confined to bed for seven weeks. For the past three days she had been delirious and semiconscious, and had had a fever. Since the early part of October she had been repeatedly passing catheters into the uterus in an attempt to produce abortion. There had been slight bleeding as a result of this instrumentation, but no pain. She had been seen by a private physician ten days before admission. At that time she was excitable, but not unconscious, and had choreiform movements of the limbs. The systolic blood pressure was 155 mm. Hg., and the pulse 130. She had been vomiting after almost every attempt to eat or drink during the past six weeks, and there had been some vaginal bleeding. Five days before admission her temperature was 99° F. There had been no chills associated with the illness. The urine at that time was negative except for a few pus cells. She had some difficulty in moving her legs, and complained of severe pain when they were touched. She had had a curettement five years before, following a spontaneous abortion at five months. There was also an abortion two and a half years ago at four months. She had three living children aged 3, 5 and 7 years.

On admission the temperature was 99° F., and the pulse 145. Respirations 28 per minute. She was semicomatose. There was a coloboma of the right eye, with loss of vision. The left fundus showed retinal hemorrhages and edema of the disc. Blood pressure 142/94. Abdominal and knee reflexes absent. Babin-ski test negative. December 31, semicomatose; temperature 102° F.; rapid pulse; pale and dehydrated. She answered questions occasionally, but in a confused and delirious manner. No facial asymmetry. Biceps and triceps reflexes were equal and normal. Abdominal, patellar, hamstring and Achilles reflexes were absent. She appeared to be using the diaphragm very little and was incontinent. She was able to raise her lower extremities to some extent. Muscles of the legs were very flaccid. Spinal puncture revealed a clear fluid under normal pressure. Urine: December 30, faint trace of albumin, no sugar. December 31, large amount of albumin and sugar and large numbers of erythrocytes (catheterized specimen). Hemoglobin 65 per cent; erythrocytes 3,560,000; leucocytes 8,100 to 11,650; 80 per cent polymorphonuclears; 20 per cent lymphocytes. January 1, labored respirations; cyanosis; temperature 103 to 105° F.; death.

Postmortem Report: Slight edema of the legs; no jaundice; no fluid in the serous cavities; heart, weight 285 gm.; liver, weight 1625 gm., cloudy swelling, no necroses; four months' fetus in the uterus.

The kidneys weighed together 280 gm. The external surfaces were smooth, the cortices cloudy.

Microscopically the glomeruli show a normal structure.

Microscopic studies of the spinal cord showed extensive degeneration and necrosis of the nerve cells (toxic myelitis).

The foregoing is an illustration of hyperemesis gravidarum in which toxic myelitis dominates the clinical picture. Berkwitz has

described several cases of this type. The hypertension and eye-ground changes suggest a relationship to eclampsia, but the liver and kidney lesions peculiar to eclampsia are not present.

There seems to be some relationship between hyperemesis gravidarum and eclampsia, but there is no convincing evidence at present that they are due to the same underlying disturbance.

5. PREGNANCY IN ASSOCIATION WITH PREEXISTING RENAL DISEASE

Eight cases have come under my observation in which it is reasonably sure that chronic renal disease was present prior to pregnancy.

CASE 21. (21-219) 26 years of age. Admitted Jan. 31, 1921. Had smallpox at age of 11 years. Frequent attacks of sore throat for many years. No history of scarlet fever or rheumatism. When she was 16 years old a physician told her she had kidney trouble. Has had headaches as long as she can remember. Her vision has been poor for years. Has noted slight pain about the heart for several years. At her first pregnancy in 1913, at the age of 18 years, she was comatose for four or five days and had convulsions. For this reason the pregnancy was terminated at the fifth month by therapeutic abortion. The second and third pregnancies ended in spontaneous abortion. The fourth pregnancy was terminated by therapeutic abortion in September, 1920. She has not menstruated since.

During her stay in the hospital the blood pressure readings were: Jan. 31, 1921, 176/140; a little later, 190/140; February 11, 122/90; February 26, 206/164; March 3, 209/160; March 14, 196/120. There was a continuous low fever. The urine was of low specific gravity, and contained a large amount of albumin. Blood urea nitrogen ranged from 89.9 to 115 mg. The phenolsulphonaphthalein test was 5 per cent on February 9. Albuminuric retinitis was present. The leucocyte count varied from 25,000 to 32,000, 80 to 90 per cent polymorphonuclears. Death May 15, 1921.

Postmortem Report: No edema; one liter of thin purulent fluid in the left pleural cavity; heart, weight 375 gm., left ventricular hypertrophy; liver, no disease.

The left kidney weighed 40 gm., and the right 25 gm.

The microscopic structure is typical advanced chronic glomerulonephritis. No glomerular lesions resembling those of Case 3.

It might be argued in this instance that the chronic nephritis resulted from eclampsia in 1913, but there was evidence of nephritis before that time and the structure of the kidney is in no way different from typical glomerulonephritis.

CASE 22. (18-237) 22 years of age. Admitted Nov. 1, 1918, in coma. She was having convulsions every hour. She had been under a physician's care for three or four months. A diagnosis of nephritis had been made, and a therapeutic

abortion performed about two months before admission. Abdominal paracentesis had been performed repeatedly. During the period of hospital observation there was a marked oliguria, and the urine contained a large amount of albumin, many casts and erythrocytes. The blood pressure was 200/120. Albuminuric retinitis was present. There was marked edema. She was in coma or semicomatose most of the time. On November 7, pericarditis was demonstrated. Blood urea was 33 mg. on November 8. Death Nov. 13, 1918.

Postmortem Report: Marked generalized edema; large amounts of thin purulent fluid in the peritoneal, pleural and pericardial cavities; heart, weight 300 gm., pericarditis only; liver, cloudy swelling; no infection in the uterus.

The kidneys weighed 130 gm. each. The external surfaces were smooth, the cortices pale and fatty.

Microscopically a typical chronic glomerulonephritis is present. There are numerous hyaline glomeruli with completely atrophied tubules. There can be no doubt that nephritis antedated pregnancy.

CASE 23. (23-267) 35 years of age. First seen April 17, 1923, complaining of weakness, dyspnea, palpitation, edema of the ankles and cough. No serious illness during childhood. Her first pregnancy resulted in a stillbirth at eight months. The second pregnancy terminated in miscarriage at six months (a macerated fetus). The third pregnancy also resulted in miscarriage. In the fourth pregnancy she developed albuminuria and convulsions, and a stillborn fetus was delivered spontaneously at eight months. The fifth pregnancy in the spring of 1919 resulted in a living child. There were postpartum hemorrhages at this labor.

She dated her present illness from August, 1922, when she developed weakness, vertigo and dyspnea on exertion. She became progressively weaker after that time. April 17, blood pressure 140/90; marked albuminuria with casts. Only a trace of phenolsulphonephthalein was excreted in two hours. Blood Wassermann negative. Signs of pneumonia appeared toward the last. Death April 24, 1923.

Postmortem Report: Edema of the lower extremities; ascites; lobar pneumonia; heart, weight 375 gm.

The right kidney weighed 60 gm., and the left 75 gm. The capsules were adherent and the external surfaces granular. The cortices were very thin.

Microscopically a typical advanced chronic glomerulonephritis is seen.

The history as well as the structure of the kidneys indicates that nephritis was present many years, and was responsible for the complications of all her pregnancies.

CASE 24. (23-435) 26 years of age. First pregnancy in 1915. Never well since that time. In January, 1919, the urine showed heavy albuminuria, many casts and erythrocytes. She complained of loss of appetite and insomnia. Shortly afterwards she became pregnant and went through pregnancy with some difficulty. She was seen at various times during the past three years, but the renal condition was not studied. She had albuminuria and slight hypertension during this period. Hemoglobin 16 per cent. Shortly before death she had repeated convulsions. Death July 10, 1923.

Postmortem Report: No edema; a small amount of fluid in the serous cavities; heart, weight 375 gm., left ventricular hypertrophy; edema of lungs; liver, normal.

The left kidney weighed 58 gm., and the right 68 gm. The capsules were adherent and the external surfaces deeply pitted. The cortices were thin.

Microscopically a typical advanced chronic glomerulonephritis is seen. In view of the history it is certain that chronic renal disease was present before the second pregnancy, but there is no history of eclampsia at any time.

CASE 25. (26-286) 26 years of age. Had scarlet fever in childhood. Numerous attacks of tonsillitis. The first pregnancy ended in abortion at two months, in October, 1922. She first came under medical care in April, 1923, during the fifth month of her second pregnancy. From April until her delivery, July 14, she had moderate edema and moderate albuminuria. The blood pressure ranged from 122/66 to 160/110. There were usually casts and erythrocytes in the urine. There were no visual disturbances and no convulsions.

She remained in the hospital three weeks after delivery. During this time the edema disappeared. The blood pressure ranged from 130/88 to 180/118. July 19, five days after delivery, the blood urea nitrogen was 57 mg., and creatinin 5 mg. August 7, nineteen days later, the blood urea nitrogen was 32 mg., and creatinin 3 mg. August 21, blood urea nitrogen was 72 mg., and creatinin 1.7 mg. Hemoglobin, July 19, 40 per cent.

The patient was observed from time to time during the next three years. The blood pressure gradually rose to higher levels. During 1925 it ranged from 180/106 to 210/130. There were no definite changes in the eyegrounds. The hemoglobin remained low. About March 19, 1926, she developed an upper respiratory infection and died four days later.

Postmortem Report: Moderate generalized edema, ascites and hydropericardium; heart, weight 380 gm., left ventricular hypertrophy; bronchopneumonia; cloudy swelling of the liver.

The kidneys weighed 90 gm. and 120 gm. respectively. The capsules were adherent and the external surfaces deeply pitted. The cortices were thin.

Microscopically an advanced chronic glomerulonephritis is found, which shows no unusual features.

The definite renal insufficiency which was found immediately after labor establishes the diagnosis of chronic glomerulonephritis at that time. Very probably it was present before the first pregnancy in 1922.

CASE 26. (23-635) 41 years of age. In September, 1919, when she was eight months pregnant she was admitted to another hospital in labor. She was delivered by forceps because she was having convulsions. The infant was still-born. She stated that she was unconscious for about one month after delivery. On admission June 8, 1921, she complained of incontinence of urine. The urine contained albumin, casts and erythrocytes. Her blood pressure was 134/90. The phenolsulphonphthalein excretion in two hours was 10 per cent. Blood urea nitrogen 34 mg. She was discharged July 20, 1921, and was not seen again until her readmission Sept. 20, 1923. At this time she was irrational and delirious. The blood pressure was 185/125. Hemoglobin 55 per cent. Erythrocytes 2,700,000. Blood urea nitrogen 125 mg. and 185 mg.; phenolsulphonphthalein output 0 per cent. Urine: specific gravity about 1010, abundant albumin, casts and erythrocytes. Slight terminal edema of the ankles. Death Oct. 3, 1923.

Postmortem Report: Slight edema of the feet; no excess of fluid in the serous cavities; heart, weight 365 gm., moderate left ventricular hypertrophy; edema of the lungs; liver, normal.

The kidneys weighed 33 gm. and 30 gm. respectively. The capsules were adherent and the external surfaces finely granular. The cortices were very thin.

Microscopically a typical, very advanced chronic glomerulonephritis is found.

Very probably the convulsions that occurred during her labor in 1919 were uremic and not eclamptic. The extreme atrophy of the kidneys indicates a nephritis of many years' duration.

CASE 27. (28-906) 37 years of age. Admitted March 26, 1928. The patient stated that she had had albumin in the urine since the birth of her last child, three and one-half years previously. Her immediate illness began in January, 1928, with dyspnea, and edema of the ankles. These symptoms gradually became more severe and she became progressively weaker until she was obliged to go to bed. The blood pressure varied from 142/104 to 162/114. The urine was of low specific gravity, and contained abundant albumin and casts. Blood urea nitrogen, March 28, 38.5 mg. May 1, hemoglobin 54 per cent; erythrocytes 3,490,000. The edema progressed until it became a marked generalized anasarca. Death July 8, 1928.

Postmortem Report: Marked anasarca; ascites; hydrothorax; heart, weight 340 gm., recurrent mitral endocarditis; liver, normal.

The kidneys weighed 73 gm. and 63 gm. respectively. The capsules were adherent, and the external surfaces finely granular. The cortices were very thin.

A typical advanced chronic glomerulonephritis is found microscopically.

The patient dated her illness from her last pregnancy, but there was no history of eclampsia. The structure of the kidneys indicates a long duration of the disease.

CASE 28. (28-123) Gravida II. 38 years of age. Admitted Jan. 18, 1928. Expected date of confinement, March 22. Said to have had a few convulsions during her first pregnancy, fourteen years before. Under treatment for hypertension for the past five years. Blood pressure usually above 200 mm. Hg., during this period. She was seen by her physician shortly after the beginning of the present pregnancy, and the blood pressure was found to be 215/120. During the course of the pregnancy the blood pressure was never found below 170/110. She entered the hospital because she was beginning to have visual disturbances, edema and vomiting. For two months she had been troubled with headaches and dizziness. During her stay in the hospital the systolic blood pressure ranged from 210 to 240 mm. Hg. There was edema of the ankles. The urine contained a large amount of albumin and many casts. Blood urea nitrogen 23.3 mg.; hemoglobin 85 per cent. During the last week she vomited almost continuously. Labor was induced January 25, and a dead fetus was delivered. The patient died suddenly about two hours after the completion of labor. There were no convulsions.

Postmortem Report: Moderate edema of the ankles; no fluid in the serous cavities; heart, weight 310 gm., moderate hypertrophy of the left ventricle; liver, weight 1700 gm., chronic passive congestion and moderate fatty metamorphosis, no areas of necrosis.

The kidneys weighed 175 and 100 gm. respectively. The smaller kidney was adherent to the surrounding tissues because of an operation several years before. The cortices were slightly cloudy.

Microscopically there is a generalized arteriolar sclerosis, but no atrophy of the parenchyma. The glomeruli show a marked thickening of the capillary basement membrane, an appearance that is found both in primary hypertension and in eclampsia.

This is undoubtedly an instance of pregnancy in a woman with preëxistent hypertension.

The foregoing 8 cases (Cases 21 to 28), illustrate the course of pregnancy in women with previous chronic renal disease. There is usually a marked aggravation of the nephritic symptoms. The distinction from gestation eclampsia is difficult when the function of

the kidneys prior to pregnancy is unknown. All the symptoms of eclampsia, namely — hypertension, albuminuria, edema, headache, visual disturbances, vomiting, convulsions and coma — may occur in nephritis uncomplicated by pregnancy. However, if a definite impairment of renal function is demonstrable, the diagnosis of pre-existing chronic nephritis may be established.

Many women with chronic renal disease go through pregnancy without serious complications, but the usual effect is an increase in the intensity of the symptoms referable to nephritis. When the uterus is emptied the nephritic symptoms usually improve, but do not disappear entirely.

There is no evidence in my experience that the presence of chronic renal disease increases the danger of the development of gestation eclampsia. Heynemann expresses a similar opinion.

DISCUSSION

In fatal cases of eclampsia and præeclampsia, a characteristic glomerular lesion is found. The glomeruli are slightly enlarged and the lumina of their capillaries are narrowed and sometimes completely closed, so that they contain very few erythrocytes. The decrease in size of the capillary lumina is caused chiefly by a marked thickening of the capillary basement membrane. Usually there is only a slight increase of endothelial cells, but sometimes the endothelial increase is prominent, and then becomes a more important factor in capillary obstruction than the thickened basement membrane.

When the increase of endothelial cells is prominent (Fig. 6), there is a definite resemblance to acute glomerulonephritis. But the more striking features of acute glomerulonephritis are absent. There are no very large glomeruli, and no epithelial crescents. Intracapillary fibers were found in only one instance, Case 16, and then in association with acute rheumatic endocarditis. Fahr noted an increase of endothelial nuclei in exceptional instances, and was inclined to attribute it to an associated infection. His view seems to be that a mild glomerulonephritis is superimposed on the eclamptic kidney in these cases. Fahr's interpretation of the endothelial increase is probably influenced to some extent by his conviction that the lesion of the eclamptic kidney is a nephrosis.

The increase of endothelial cells is surely an inflammatory phenomenon, that is, a form of glomerulonephritis. It occurs so frequently that Fahr's interpretation of superimposed infection seems doubtful. A simpler explanation is to consider it a reaction to the toxic substance responsible for the toxemia of pregnancy.

The interpretation of the thickening of the capillary basement membrane is more difficult. It is not clear whether this is a degenerative or an inflammatory phenomenon. One is tempted to explain it as a compensatory reaction to increased intravascular pressure since it occurs so regularly in primary hypertension. But it was found in one case of hyperemesis gravidarum (Case 19) in which there was no elevation of blood pressure, and I have found it in instances of lipoid nephrosis without hypertension.

Volhard's theory of arteriolar spasm does not seem adequate to explain thickening of the capillary walls. We are left with the theory that a soluble toxic substance in the blood is responsible for the thickening of the basement membrane. This view can neither be established nor disproved with our present knowledge.

The thickening of the capillary basement membrane is apparently a process of hypertrophy, similar to the thickening of the elastica interna of the arteries that occurs in advanced life. Whether this is an inflammatory process or not depends upon how inflammation is defined. It is not what one ordinarily understands as inflammation, but it probably represents a reaction to some kind of stimulus.

The glomerular lesion of eclampsia is, therefore, a distinct pathological entity. It is perhaps best classified as a special form of glomerulonephritis, although it is to be distinguished from ordinary glomerulonephritis. It is somewhat confusing to classify a disease of this type as nephrosis.

The classification of the lesion as nephritis or nephrosis is of no great importance. The essential nature of the disease is an injury of the glomerular capillaries, which allows albumin to escape and impedes the flow of blood through the kidneys.

The glomerular lesion may be the primary cause of the hypertension rather than its effect. The fact that hypertension often precedes albuminuria does not exclude this interpretation, since the narrowing of the capillaries may take place before they are permeable to albumin.

The glomerular lesions support the view that the fundamental

cause of eclampsia is a toxic substance in the circulating blood. The peculiar histological structure suggests that the poison is quite distinct from that causing glomerulonephritis.

SUMMARY

In fatal cases of eclampsia a characteristic glomerular lesion is found.

The glomeruli show a marked narrowing of all their capillaries, caused usually by an increase in thickness of the capillary basement membrane, but sometimes by an increase of endothelial cells.

One case is reported (Case 3) in which the lesions resulting from an attack of eclampsia seven years before are described. These consist of focal hyaline areas in the glomeruli with partial or complete glomerular obliteration and varying degrees of tubular atrophy. It is shown that a peculiar form of chronic renal disease may result from the eclamptic kidney.

In one case of hyperemesis gravidarum (Case 19) glomerular lesions were found which correspond entirely to those of typical eclampsia. In three other cases the glomeruli were normal. A fatty liver without necroses is characteristic of this form of toxemia.

When a woman with chronic renal disease becomes pregnant, there is usually an aggravation of all the nephritic symptoms. The condition cannot be distinguished from preëclampsia and eclampsia unless the condition of the kidneys prior to pregnancy is known, or unless there is a definite impairment of renal function. Chronic nephritics show no special tendency to develop gestation eclampsia.

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DESCRIPTION OF PLATES

PLATE I

FIG. 1. Case 14. Photomicrograph of glomerulus under low magnification, showing extensive obstruction of the capillaries. Hematoxylin-eosin stain.

FIGS. 2, 3 and 4. Case 3. These photomicrographs illustrate the effect of an attack of eclampsia seven years before death. The hyaline glomeruli are associated with atrophic tubules (Fig. 2). Figures 3 and 4 show partially obliterated glomeruli.



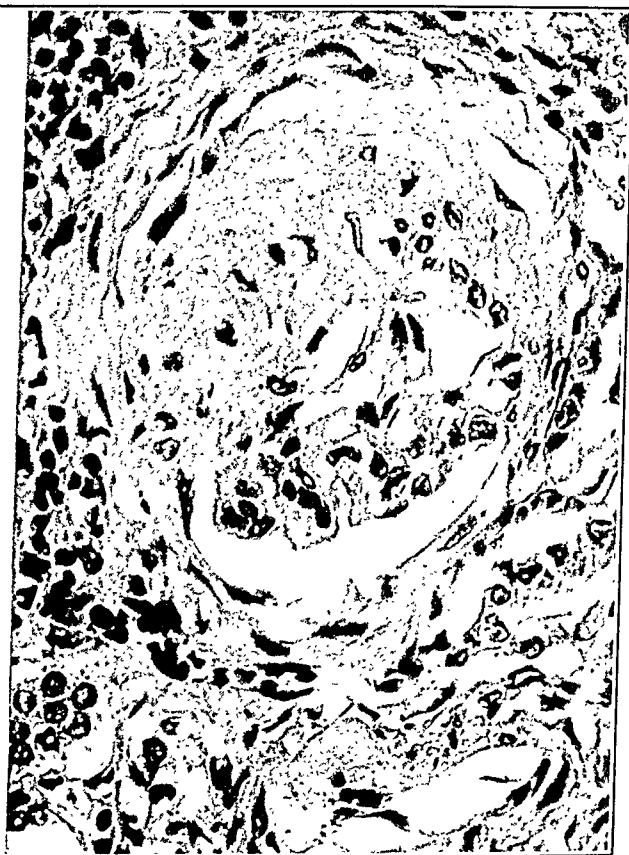
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PLATE 2

FIG. 5. Case 14. Drawing showing portion of a glomerulus under high magnification. Azo-carminc stain. Note the marked thickening of the capillary basement membrane (b. m.). End., endothelial cell; ep., epithelial cell; g., hyaline granules in an epithelial cell; er., erythrocyte; l., lumen of capillary.

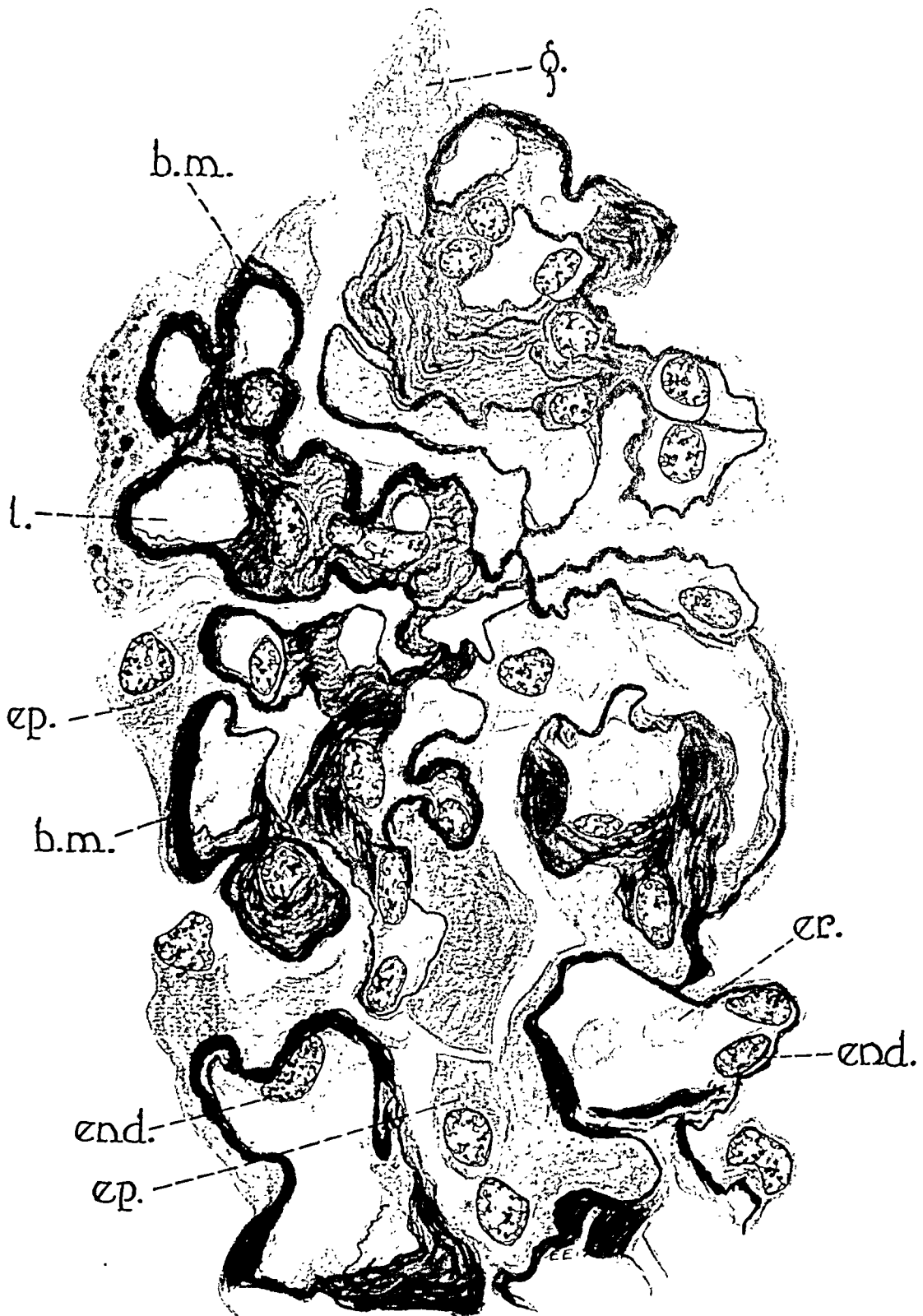
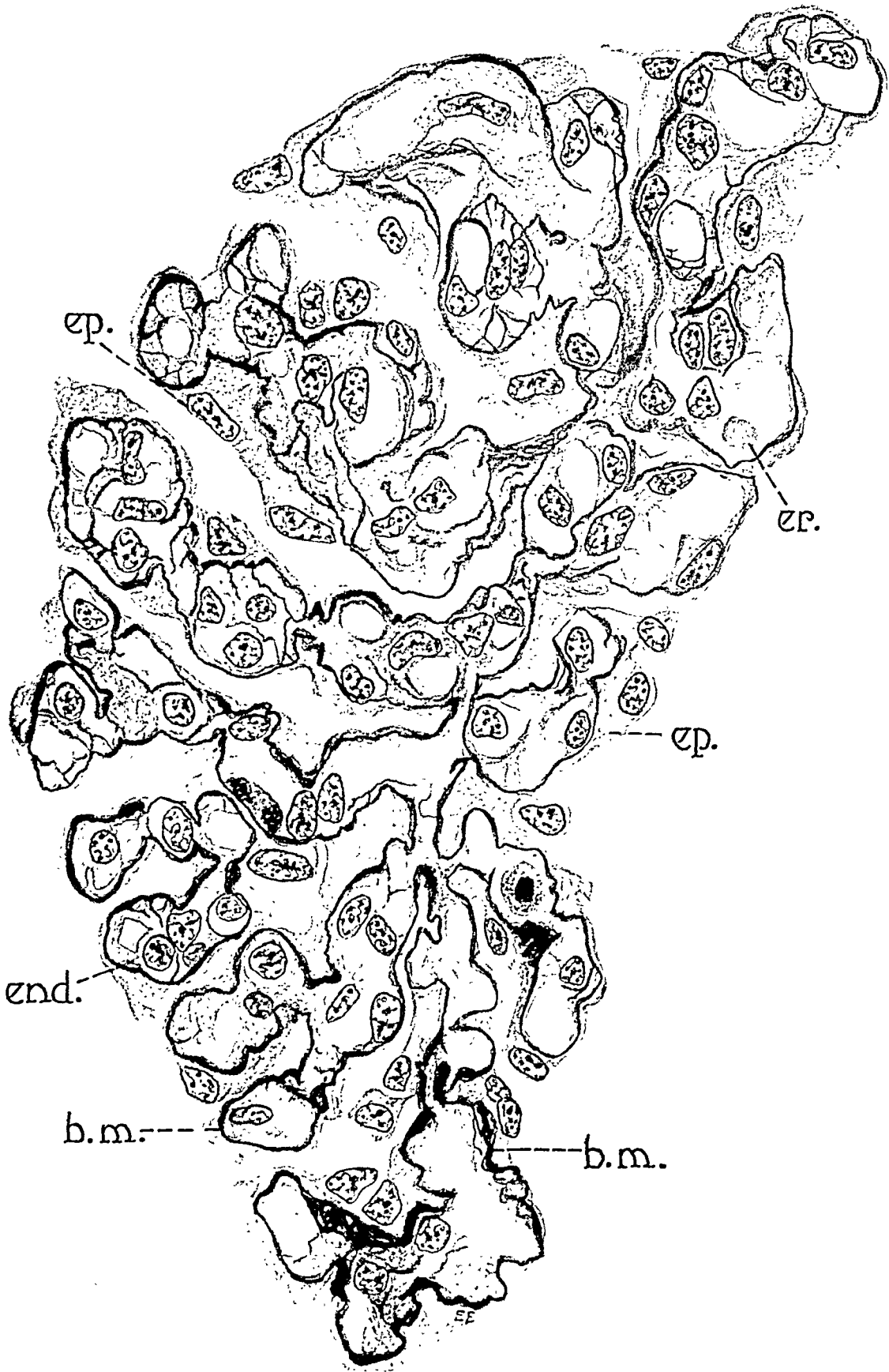


PLATE 3

FIG. 6. Case 11. Drawing showing portion of a glomerulus under high magnification. Azo-carminc stain. Note the marked increase of endothelial cells (end.). The capillary basement membrane (b. m.) shows a moderate but uneven thickening. Ep., epithelial cell; er., erythrocyte.



INFECTIOUS ORAL PAPILLOMATOSIS OF DOGS *

W. A. DEMONBREUN, M.D., AND E. W. GOODPASTURE, M.D.

(From the Department of Pathology, Vanderbilt Medical School, Nashville, Tenn.)

For many years it has been the general opinion that human warts are contagious. The first experimental evidence of their infectious nature was furnished by Variot ¹ (1893) who reported the successful inoculation of an adult with material from warts obtained from a child. Jadassohn ² (1894) added confirmatory evidence when he succeeded in inducing the formation of typical warts in thirty-one out of seventy-four transplants of wart tissue under the epidermis. He reported the incubation period to be from seven to twelve weeks. Lanz ³ (1899) and Juliusberg ⁴ (1903) reported the successful transmission of warts, giving the incubation periods as six weeks and fifty days respectively. Ciuffo ⁵ (1907) first succeeded in passing the infectious agent through a Berkefeld N filter. Its filterability was confirmed by Serra ⁶ (1908) who used a Berkefeld W candle. Wile and Kingery ⁷ (1919), by the use of Berkefeld filtrates, were able to produce warts in four weeks. Later Kingery ⁸ (1921) succeeded in carrying the warts to the second generation by the use of Berkefeld filtrates, the incubation period being about six months. Findlay ⁹ (1930) found that the time required for Berkefeld filtrates to produce the lesions varied from three to eight months.

Most of the experimental work with warts has been done with the common wart (*verruca vulgaris*), which is so frequent in children. Other varieties such as plantar, digitate, and filiform warts are also probably caused by the same virus or one similar to that which causes common warts, and, as has been pointed out by Roxburgh ¹⁰ (1928), their differences in structure are due mainly to the characteristics of the situations in which they occur. Their papillomatous structure tends to be more pronounced in the moist, warm areas. In support of this opinion is the report of Waelsch ¹¹ (1918) who inoculated healthy skin and mucous membranes with *condylomata acuminata* and obtained flat warts on the skin and papillomas

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on the mucous membranes. Serra ¹² (1924) and Findlay ⁹ (1930) also report the filterability of the infectious agent of condylomata acuminata.

Numerous observers have noticed that children with laryngeal papillomas frequently have warts on their hands. Ullmann ¹³ (1923), having noticed this association, successfully inoculated the human skin with macerated material from a laryngeal papilloma removed from a child. He succeeded in carrying the papilloma to the third generation, the virulence of the virus being definitely increased by the passage. In addition to demonstrating the filterability of the infectious agent, Ullmann also claims to have infected the vaginal mucous membrane of a dog, although he was not able to induce lesions in the dog's hard palate, or abdominal skin. Findlay ⁹ (1930) was not able to confirm Ullmann's claim of a successful inoculation of a dog with human material.

Several of the lower animals are subjects to warts. Magalhães ¹⁴ (1920) reports that the infectious agent of bovine warts is filterable, lesions appearing five weeks after the injection of the virus. Sanfelice ¹⁵ (1913) describes a papillomatous condition of the frog, *Discoglossus pictus*; but apparently no studies were made regarding its infectiousness. Borst ¹⁶ describes pointed condylomas which occur primarily on the genitals of dogs, and secondary warts on the lips due to the animals' habits of licking.

Penberthy ¹⁷ (1898) described an epidemic of mouth warts in otherwise healthy foxhound puppies. Only three out of forty puppies in the kennel escaped the disease. The similarity of the growths to human warts, as regards their spontaneous disappearance, was noticed. M'Fadyean and Hobday ¹⁸ secured two of Penberthy's wart-bearing puppies for experimental purposes. By tissue inoculation they succeeded in transferring the warts to the scarified buccal mucous membranes of other puppies through the second generation. The incubation periods varied from one month to six weeks. Subcutaneous inoculations of wart emulsions failed to produce the new growths. An unsuccessful attempt was made to infect the mucous membrane of the penis of one puppy. In most cases the warts disappeared spontaneously after about six weeks. One puppy that had recovered resisted reinfection successfully.

While our studies of oral papillomas of dogs was in progress there came to our attention a statement by Findlay ⁹ (1930) that

this disease may be propagated in series by means of a Berkefeld filtrate.

Several writers have described cellular inclusions in association with wart lesions. As early as 1913 Sanfelice¹⁵ described eosinophilic intranuclear inclusions in the prickle cells of warts in *Discoglossus pictus*. Lipschütz¹⁹ (1924) describes basophilic intranuclear inclusions in cells of the outer portions of the prickle cell layer of human warts. In sections of warts fixed in Schaudinn's fluid and stained by Giemsa's method, Ullmann¹³ (1923) recognized both red and blue inclusions in the nuclei of cells within the prickle layer. He states the inclusions are found in only certain stages of the disease, being more common in human than in dog warts. These inclusions have been regarded by Kyrle²⁰ (1925) as an oxychromatic degenerative change of the nuclei. On the other hand Sangiorgi²¹ (1915), in studying human warts, found only cytoplasmic inclusions which appeared as single or multiple eosinophilic bodies in certain cells of the prickle layer. He discussed the possibility of these bodies being keratohyaline masses, but concluded that they must have some relation to the disease.

Infectious oral papillomatosis in dogs first came to our attention during the course of other experiments which required that the animals be observed for rather long periods of time. A young, almost fullgrown, male setter that had been under our observation for almost two months was noticed to have several papillomatous growths varying from 2 to 4 mm. in diameter located near the inner margins of the upper lips. These pedunculated, cauliflower-like growths were grayish in color, being considerably paler than the surrounding mucous membrane. A few days later several other comparatively smooth, grayish nodules were noticed scattered over the mucous membrane of the lips. The surfaces of these new nodules gradually became roughened, and in about two weeks they were definitely papillomatous in character. The warts increased in size and numbers, spreading to the lower lips, both surfaces of the tongue, hard palate and pharynx. The nasal and genital mucous membranes and the skin remained free of abnormal growths. Following about three months of active growth, the warts seemed to remain stationary for a period of another three months. Removal of some of the warts for experimental purposes from time to time during the period of observation might have had some bearing on their disappearance,

which was complete within three weeks after regression had commenced.

In satisfying our requirements for experimental animals a brown setter bitch, likewise bearing oral warts, came into our possession. This animal was noticed to have a peculiar laryngeal stridor which we thought might be due to the presence of one of the polypoid growths in the larynx. Autopsy of the animal, however, showed the larynx to be free from abnormal growths, and although numerous warts were present on the posterior pharyngeal wall, and a few small ones on the anterior surface of the epiglottis, none was encountered below the level of the glottis. The gastro-intestinal canal below the pharynx, as well as the genitalia, likewise proved to be free from warts.

The infectiousness of the disease was proved by inoculating the scarified buccal mucous membranes of puppies with a paste made by grinding pieces of the wart tissue in a mortar containing small amounts of sterile sea sand and physiological saline solution. After thoroughly rubbing the paste into a scarified area, the mucous membrane was allowed to dry about five minutes before the animal was freed. At first the inoculations were repeated on the following day, for it was thought that it might be necessary for the infectious agent to come in contact with previously damaged cells in order to produce the disease. Later, one inoculation was found to be sufficient to induce the lesions in healthy puppies. In this manner the disease was carried to the fifth generation, the incubation period being from thirty to thirty-five days, with a tendency to be somewhat longer in dogs in poor general condition. Regression of the warts usually became apparent between the third and fifth months following inoculation.

The scarified abdominal skin of puppies, guinea pigs, rabbits, rats, and mice was inoculated without result. With the exception of the puppies, lesions could not be induced in the buccal mucous membrane of any of these animals. Monkeys (*M. rhesus*) and kittens likewise proved unsusceptible. The buccal mucous membranes of four old dogs were inoculated, but in none of them did warts develop. On account of the immunity which many older dogs apparently had, practically all of our experimental work was done with puppies. We have noticed, however, the development of mouth warts in several young adult dogs following contact with infected

puppies. In these cases the incubation periods varied, in so far as we could judge, from thirty to thirty-five days, being approximately the same as in cases where the mucous membranes were scarified and inoculated directly with infectious material.

The wart lesions are easily propagated in series by means of the Berkefeld filtrate. In preparing the filtrate, the fresh warts were removed and washed free of adherent blood clots with Locke's solution. They were then pressed between layers of filter paper to remove the excess moisture, after which they were weighed. Next, they were placed in a mortar containing a small amount of sterile sea sand, and ground to a paste. Sufficient Locke's solution was gradually added to make the resultant emulsion contain approximately 5 per cent of tissue. The emulsion was then allowed to stand overnight in the icebox, after which the grinding was resumed for about five minutes. The contents of the mortar were then centrifuged at low speed for ten minutes, in order to throw down the larger particles of tissue. After adding a small quantity of a 24 hour broth culture of *B. prodigiosus*, the supernatant fluid was passed through a Berkefeld N candle at 20 pounds pressure per square inch. Cultures of the filtrate were sterile.

Using very small hypodermic needles, the sterile filtrate was injected into the mucous membranes of the lips of three female puppies. The injections were made so as to form three or four discrete, white blebs, each containing approximately 0.1 cc. of the filtrate. The vaginal mucous membranes were likewise inoculated. Also, intradermal injections of the filtrate were made into the shaved abdominal skin of each puppy. No inflammatory reactions appeared about any of the sites of inoculation. After the third week the animals were examined daily. On the thirtieth day following the inoculations there were noticed in one puppy, minute, pale, slightly elevated areas at the sites of inoculation in the buccal mucous membrane. Their subsequent development proved them to be warts in the earliest noticeable stage. Three days later similar lesions were noticeable in the mouths of the other two puppies. Also at this time early lesions became demonstrable in a positive control puppy whose buccal mucous membrane had been scarified and inoculated with sediment obtained by centrifugation of the tissue emulsion during the process of preparing the filtrate.

Lesions failed to develop at the sites of inoculation in the skin and

vaginal mucous membranes. Cutaneous and vaginal injections were later repeated, using a filtered virus, but with similarly negative results. In addition, the conjunctivas of three puppies proved unsusceptible to the disease following injections of the virus, although injections made into the buccal mucous membranes of these puppies produced typical lesions.

The warts were carried to the third generation by means of Berkefeld filtrates prepared as previously outlined. By this process, the incubation period was not appreciably shortened. In one experiment the filtrate was collected in two fractions at 20 pounds pressure per square inch. Each fraction seemed to be about equal in virulence, for, when injected into the buccal mucous membranes of healthy puppies, each produced apparently identical lesions following incubation periods of equal lengths. A filtrate which showed no apparent diminution in virulence was likewise obtained by using a Berkefeld W candle.

Some of the warts were removed and immediately frozen with "dry ice" and kept in the frozen state while being dried over phosphoric anhydride *in vacuo* at a pressure of 2 mm. of mercury. Examination of the material after forty-eight hours showed it to be thoroughly dry. It was then sealed in glass ampoules and stored in the icebox. Sixty-three days later a portion of the dried material was emulsified in saline and inoculated into the scarified buccal mucosas of two young puppies. In the mouths of both puppies typical warts began to be apparent on the thirty-second day following the inoculation.

The virus may also be preserved in equal parts of glycerol and 0.9 per cent NaCl solution. Several warts were removed and immediately placed in the glycerol-saline solution, and kept in a refrigerator at about 10° C. Sixty-four days later a portion of the preserved tissue was rinsed in several changes of normal saline and then ground to a paste in a mortar. The buccal mucous membranes of two puppies were scarified and inoculated with this paste. One puppy died three weeks later without showing any signs of warts. In the other, warts began to be noticed on the thirty-third day following inoculation.

The virus is not killed by a temperature of 45° C for one hour. A Berkefeld filtrate was prepared as previously described. Portions were sealed in each of three ampoules. The samples were heated for

one hour in water-baths at temperatures of 45° C, 58° C, and 80° C, respectively. Each sample of virus was then injected into the buccal mucous membranes of two puppies. Unheated virus was also injected into the buccal mucous membranes of two other puppies as controls. Warts first became noticeable in the control puppies on the thirty-seventh day. None of the other puppies developed warts save those injected with virus which had been heated to 45° C. In one of these animals the incubation period was thirty-eight days, in the other forty-two days. It is believed that the prolonged incubation periods of the disease in the experiment were due almost entirely to the poor general condition of the animals caused by the extremely hot weather which existed during the course of the experiment.

It has been our experience that following inoculation, young healthy puppies develop the disease quicker than undernourished puppies, especially those with intercurrent disease. One fat bull puppy developed lesions in twenty-two days following the injection of the filtrate. Not uncommonly the appearance of the lesions may be delayed a week to ten days in sickly puppies. The average incubation period is from thirty to thirty-three days. There is evidence that practically all puppies are susceptible to the disease. Out of thirty-four puppies inoculated with the virus, all save nine developed the lesions, and every one of these exceptions became sickly soon after inoculation, and all died before the upper limit of the incubation period had been reached.

Very little is known about the susceptibility of older dogs. We have noticed several instances where older dogs developed lesions following contact with infected puppies. On the other hand, other dogs, after repeated contact with infected puppies, did not develop the lesions.

Attempts were made to reinfect four puppies in which complete regression of the lesions had taken place. In no instance were we successful. Two other puppies, each bearing lesions of about thirty days' duration on one side of an upper lip, were reinoculated with virus on the opposite sides. Neither developed fresh lesions.

The gross appearance of the lesions varies considerably throughout the course of the disease. In case the buccal mucosa has been scarified and inoculated with the virus, the lesions usually become noticeable around the thirtieth day following inoculation as pale, smooth elevations which correspond to the lines of scarification.

At the end of a week the elevations are more conspicuous, and their surfaces tend to be slightly rough. As a rule their growth during the second week seems much greater than during the first week. At first the lesions are flat, but by the end of the second week they begin to assume their characteristic papillomatous character. The older warts consist of a mass of closely packed papillae which are often as much as 7.5 mm. in length. If the lines of scarification are made close together during the inoculation process, a confluent mass of warts results. In case the lips are infected by injecting the virus into the buccal mucosa, the first visible lesions appear as minute, pale spots which correspond to the sites of the blebs made at the time of injection. These spots soon become pale elevations, which, as growth goes on, frequently assume a lobulated character due to unequal growth in different areas. These, too, soon assume their characteristic papillomatous conformation. The early lesions always appear paler than the mucous membrane which surrounds them. In case they are growing on pigmented lips the color contrast is very noticeable; although the warts may have a slaty gray pigmentation if they arise on dark mucous membranes.

Regressing warts usually become darker in color and somewhat shriveled. They gradually become smaller, regression usually being complete within two weeks of its commencement. The healing process leaves no scars. After the lesions have existed for four to six weeks, secondary warts in other parts of the mouth have not infrequently been observed.

The general histological characteristics of the lesions are very similar to those of human warts. The very early lesions show extensive hyperplasia of the epithelium, as indicated by numerous mitotic figures. As a result the prickle cell layer grows progressively thicker. In lesions a little older, there may be seen small islands of rounded prickle cells which seem to have lost their intercellular bridges. These groups of cells evidently represent the beginning of the formation of papillae. As further development takes place hyperkeratosis becomes a prominent feature. The cells of the Malpighian layer remain approximately normal in size. Many of the squamous cells increase to several times their normal size and have intensely staining nuclei. The cytoplasm of many of them appears as coagulated fluid; other cells appear vacuolated. In the outer portions of the squamous cell layer the nuclei appear shriveled, and many of the cells have en-

tirely disappeared, leaving mesh-like spaces in the thickened layer of keratin. During the development of the lesion the corium presents no abnormal cellular changes except hyperplasia to form papillae. In a few of our sections showing a wart that is definitely degenerating there may be seen a few round cells and plasma cells in the corium.

It is only in sections of older warts that any suggestion of cellular inclusions is to be seen. In such sections many of the cells just beneath the keratin layer are seen to contain within their cytoplasm round to oval bodies which vary from 1 to 5 microns in diameter. These bodies may be seen sometimes in the swollen squamous cells, but more commonly they occur in the cells of normal size. These bodies are usually acidophilic, presenting the same staining reactions as the adjacent keratin. They are regarded as keratohyaline masses. Also, within the nuclei of a few of the swollen cells just under the keratin layer there may be seen large round to oval, intensely basophilic masses which almost fill the nuclei. Occasionally lobulated intranuclear masses may be seen. They are never observed except in areas where the cells are definitely degenerating. Stainable granules which might be interpreted as representing the virus could not be identified in smears of wart tissue stained according to the technique of Morosow.

DISCUSSION

The virus of oral warts in dogs seems to possess greater cellular specificity than does the virus of human warts. This is indicated by our success in inducing lesions only in the mouths of puppies, although the skin and other mucous surfaces were likewise injected with the virus. Further evidence of the specificity of the virus is furnished by our failure to induce the lesions in the buccal mucous membranes of kittens, rabbits, guinea pigs, rats, mice and monkeys. In view of the remarkable specificity of the wart viruses we believe that confirmation should be obtained before Ullmann's report of the successful inoculation of a dog's vaginal mucous membrane with human wart virus is generally accepted.

Little is known about the proportion of older dogs that are susceptible to the disease, but judging from our experiments practically all healthy puppies are susceptible. The average incubation period is from thirty to thirty-three days. In malnourished or sickly puppies

frequently another week is required before the lesions become apparent. We are unable to explain this variation, but such factors as malnutrition, acidosis, dehydration, or elevated temperature may play a part in retarding the development of the lesions.

The experimental lesions have a tendency to regress somewhat earlier than the spontaneous ones. In some cases the warts had completely disappeared within six weeks after their appearance. Surgical removal of portions of the warts, for experimental purposes, no doubt hastened their regression. Animals in which warts were definitely regressing could not be successfully reinoculated with the virus, although the regressing warts still contained active virus capable of infecting normal puppies. This fact suggests that the development of immunity determines the limit of growth and initiates the regression of the papillomas.

In microscopic sections of younger lesions we have been unable to observe anything convincing of virus inclusions. Older lesions show in some of the cells of the peripheral portions of the prickle layer small, rounded, acidophilic, intracytoplasmic masses which we judge to be masses of keratohyalin. In an occasional large, characteristic wart cell there may be seen a large basophilic body which almost completely fills the nucleus. These homogeneous, basophilic structures are quite similar to the Lipschütz bodies encountered in human warts. On the basis of our present knowledge we regard them as abnormal degenerative products of the nuclei occurring secondarily to the processes of hyperplasia and hyperkeratinization.

Notwithstanding the absence of definite cellular inclusions which suggest to us the presence of an intracellular virus, we recognize in warts, both human and canine, cellular changes which we regard as characteristic of the infection. These consist mainly in an enlargement of certain epithelial cells, associated with an increase in both cytoplasm and nucleus. Not infrequently the apparent volume of the cytoplasm is relatively more greatly increased than the nuclear volume. This change results in the appearance of large clear epithelial cells, singly or in small groups, surrounded by normal cells. The altered cells do not differentiate but tend to remain simple and intact even after keratinization has completely immured them.

This characteristic cellular change indicates to us that the virus of warts is an intracellular agent, that is, a cytotropic virus. It is noteworthy that occasionally a large "wart cell" appears to rupture,

leaving a small cyst-like space filled with serum. Like certain other cytotropic viruses, that of warts excites cells to hyperplastic growth, and leads to abnormal structural changes which may eventuate in swelling and lysis or rupture. But above all, the lesion is characterized by localized hyperplasia.

The ease with which the virus passes through the finest Berkefeld candle suggests the likelihood that it will also pass through still finer filters such as the Chamberland.

Several qualities contribute to make this virus ideal for the study of its physical properties. Among these are its remarkable specificity, the ease with which it may be secured in quantity, the ease of its preservation by drying (vacuum or glycerine), the susceptibility of practically all puppies to infection by it, and the fairly constant incubation period of the disease which it produces.

Puppies recovered recently from the experimental disease are immune to subsequent inoculation.

SUMMARY

1. Infectious papillomas occurring in the mouths of dogs have been described.
2. The general histological characteristics of the lesions are very similar to those of human warts.
3. Basophilic intranuclear bodies, similar to the Lipschütz bodies of human warts, occur in a few of the large wart cells of the older lesions. Their connection with the etiological agent of the disease remains to be proved.
4. Judging from our experiments practically all puppies are susceptible to the disease, but little is known regarding the proportion of older dogs that are susceptible.
5. The average incubation period of the disease in healthy puppies varies from thirty to thirty-three days, but may be as much as ten days longer in malnourished, sickly puppies.
6. The lesions usually heal spontaneously. Regression in the experimental lesions occurs somewhat earlier than in the natural infection.
7. Puppies that have recovered from the disease are immune to reinfection.

8. We have not succeeded in inducing the disease in rabbits, rats, mice, guinea pigs, kittens, or monkeys.

9. In puppies the disease is easily transmitted in series by means of Berkefeld filtrates obtained from the lesions.

10. The virus possesses a high degree of cellular specificity, apparently affecting only the mucous membranes of the mouth.

11. The virus may be preserved for long periods in equal parts of glycerol and saline, or by drying the infectious tissue *in vacuo* while frozen.

12. Subjection of the virus to a temperature of 58° C for one hour renders it non-infectious. A temperature of 45° C for a similar period does not appreciably impair its virulence.

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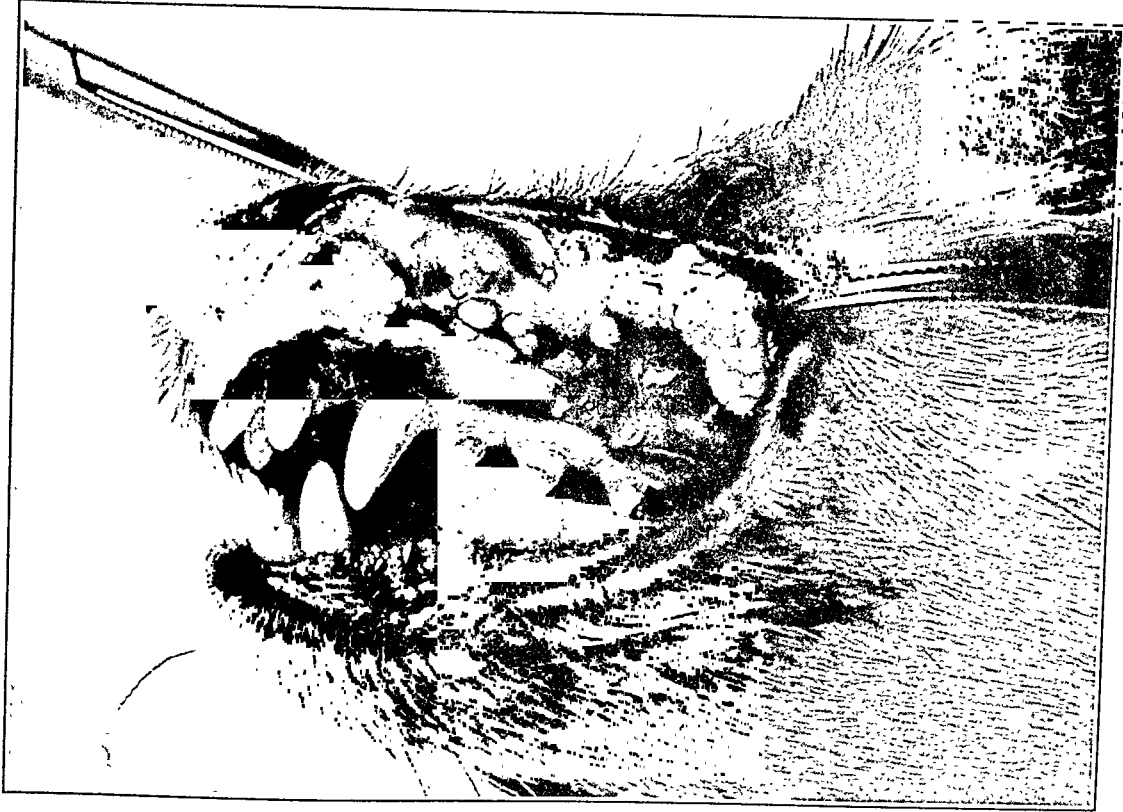
DESCRIPTION OF PLATES

PLATE 4

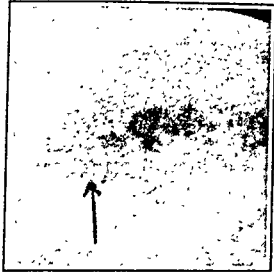
- FIG. 1. Mouth of dog showing spontaneous warts. This photograph was made twelve weeks after the first small group of warts was noticed.
- FIG. 2. Puppy's mouth showing the warts as they appeared sixty-six days after injection of the Berkefeld filtrate. In this case the incubation period was thirty-three days.
- FIG. 3. Photograph of very early lesions as they appeared following inoculation of the scarified buccal mucosa. Note the linear arrangement of the lesions corresponding to the lines of scarification.
- FIG. 4. A cross-section of the lesion shown in Fig. 3. Note the epithelial hyperplasia. $\times 60$.



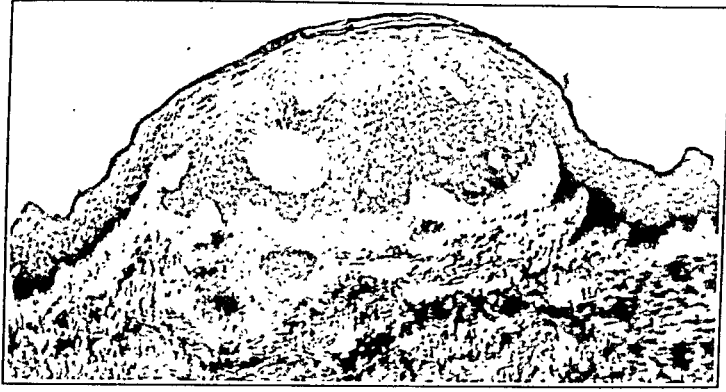
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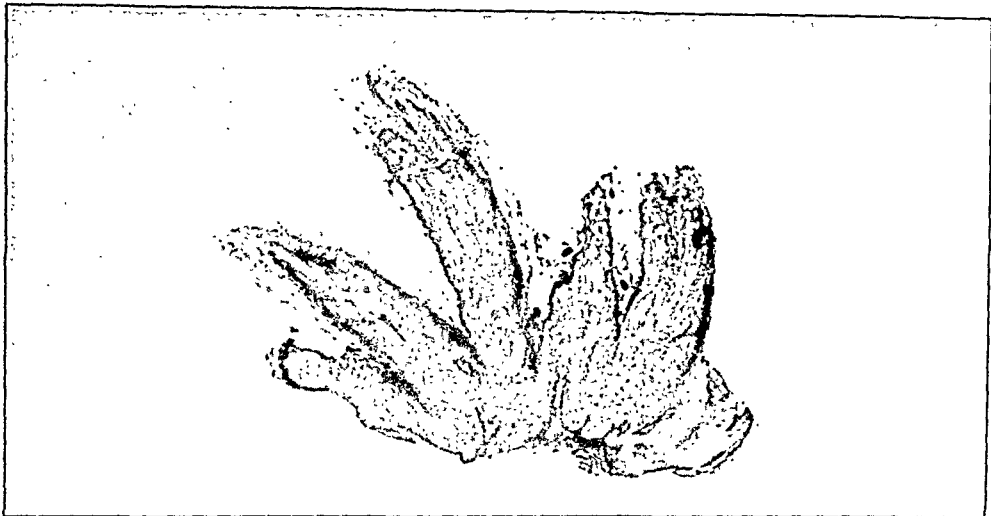
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PLATE 5

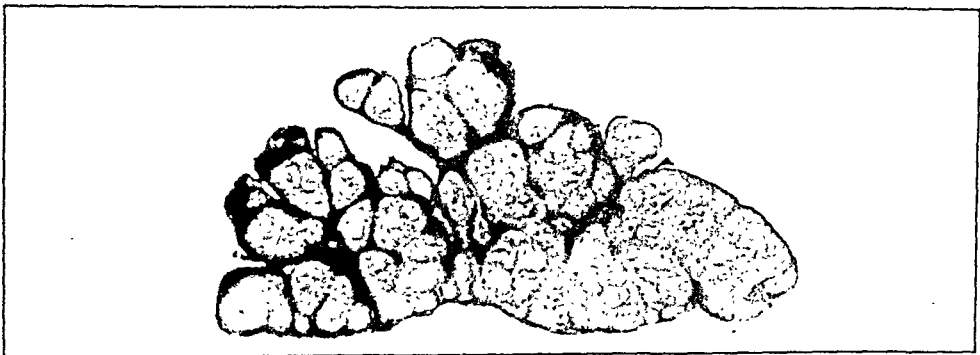
FIG. 5. Longitudinal section of an older wart showing its papillomatous nature. $\times 12$.

FIG. 6. Transverse section from an old wart. $\times 12$.

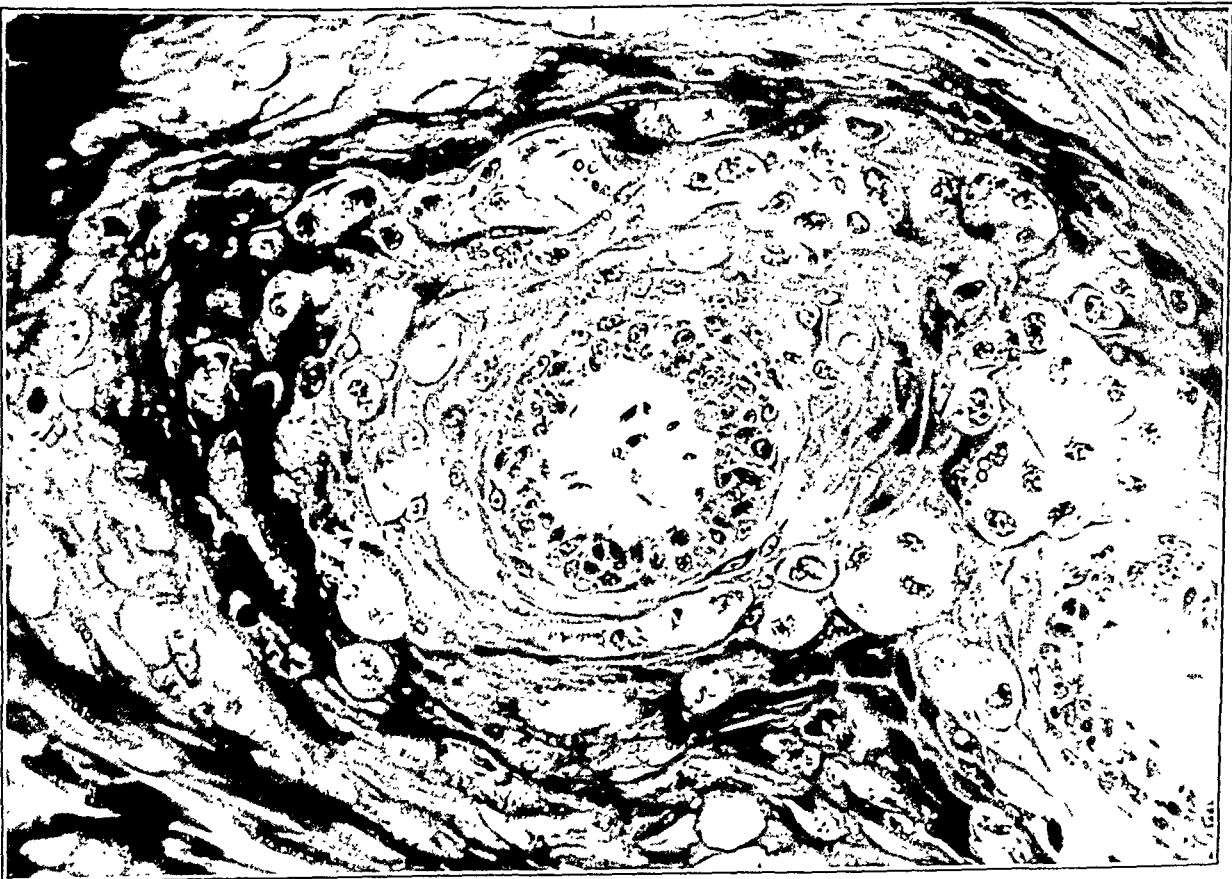
FIG. 7. A higher magnification of a transverse section of a papilla of an actively growing wart. Note the inner core of malpighian cells which are approximately normal in size. Surrounding these may be seen the large, characteristic wart cells. $\times 300$.



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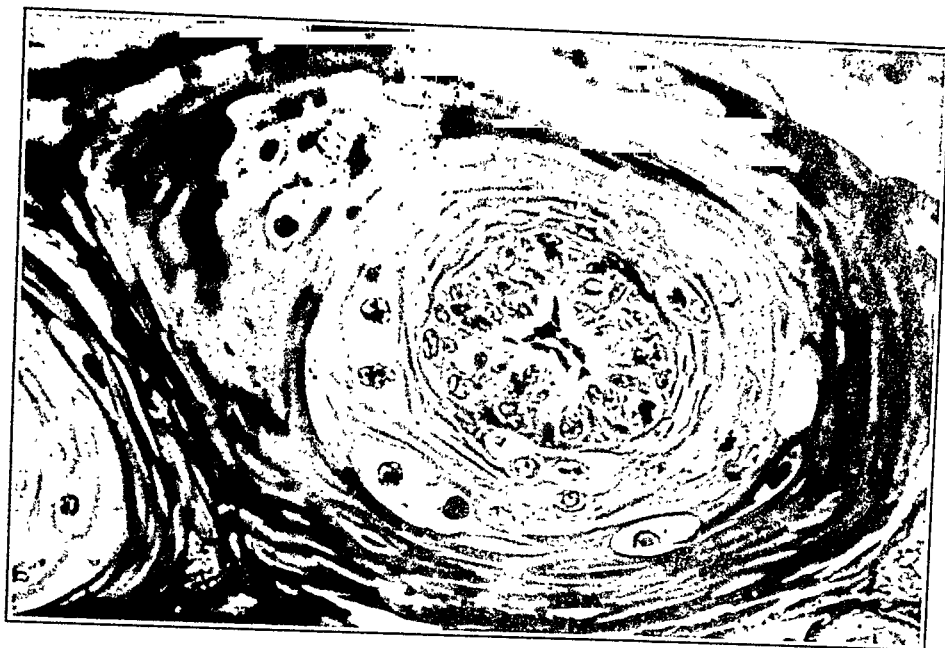
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PLATE 6

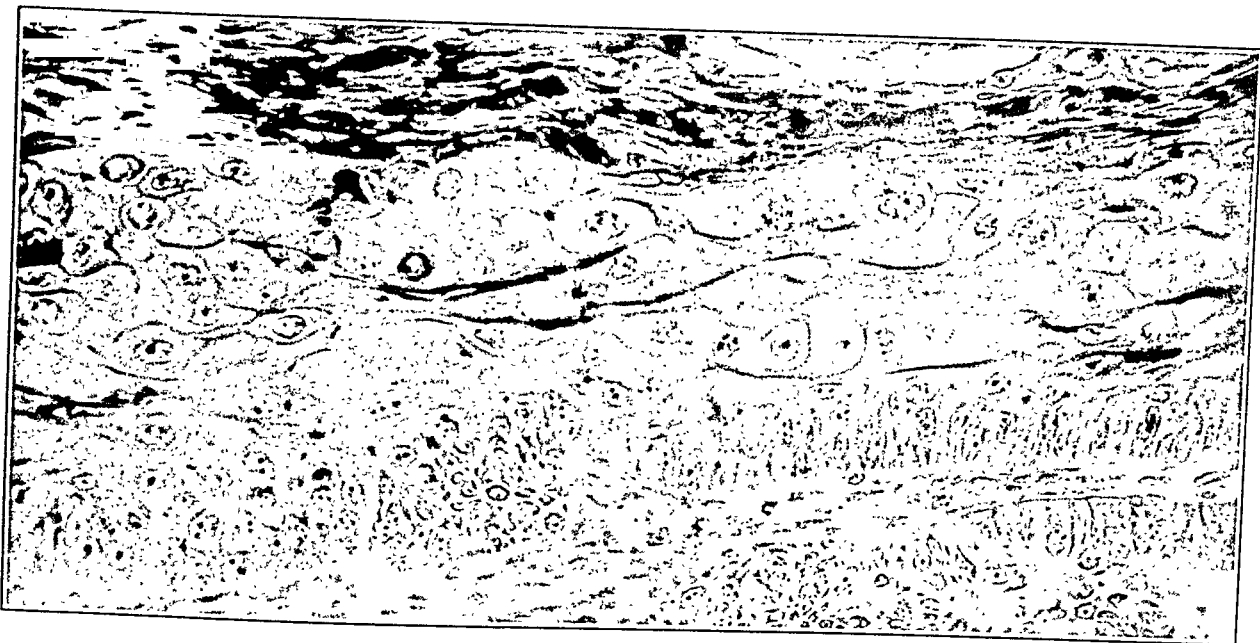
FIG. 8. Cross-section of a papilla showing marked keratinization. $\times 300$.

FIG. 9. Longitudinal section of a papilla showing the large wart cells lying between the malpighian cells and the outer layer of keratin. $\times 300$.

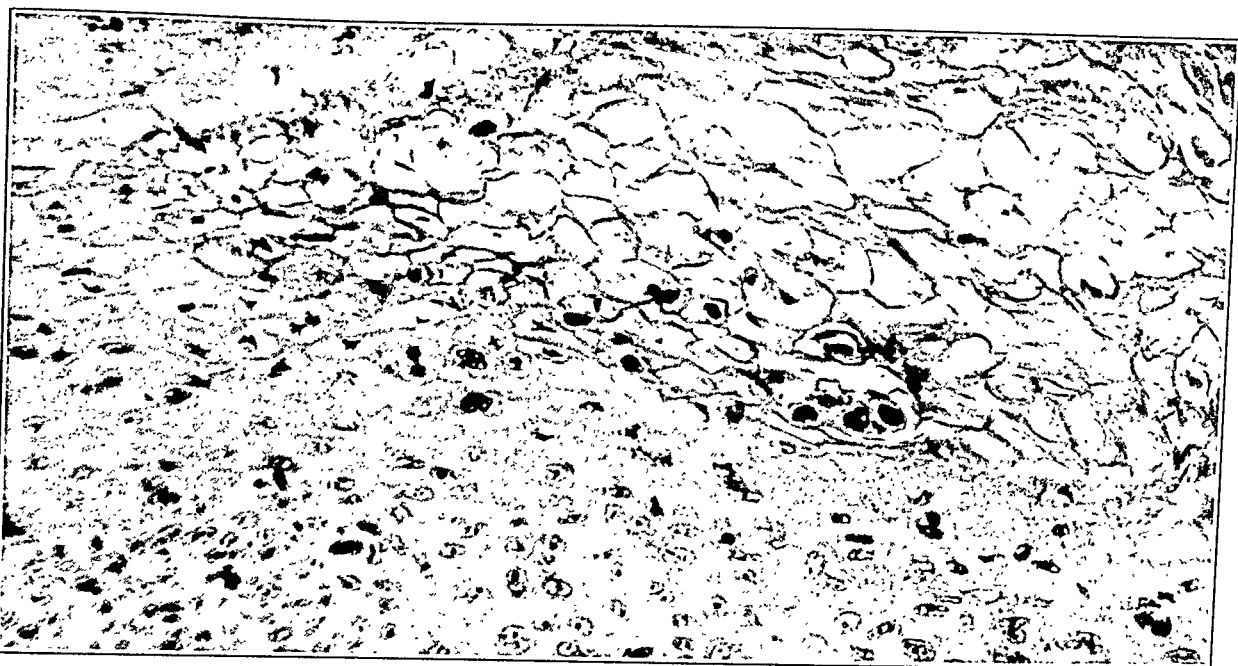
FIG. 10. Longitudinal section of an older wart showing an area from which many of the large wart cells have disappeared, leaving a mesh-like arrangement of the keratin. $\times 300$.



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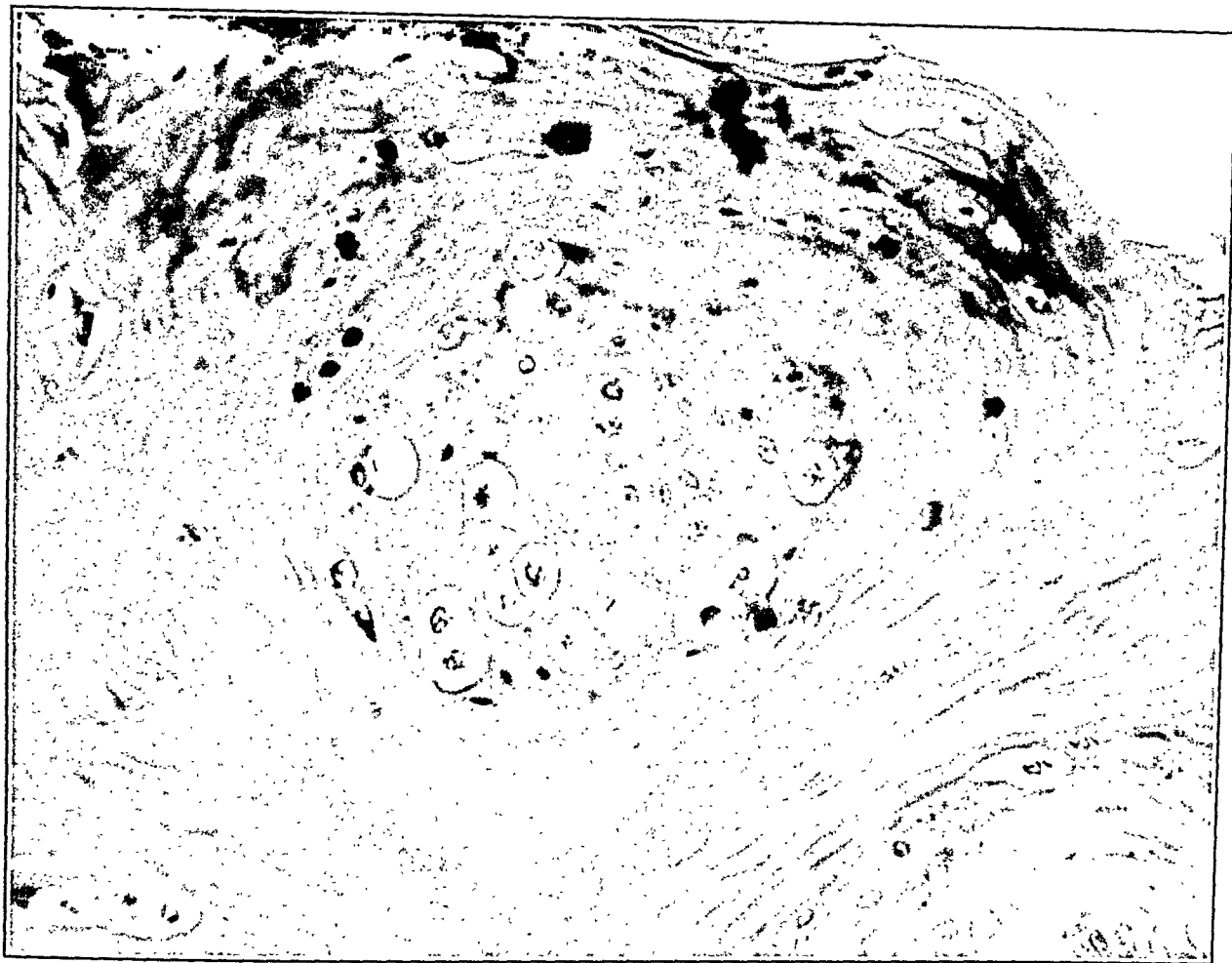


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PLATE 7

FIG. 11. Cross-section of a papilla from a regressing wart. Malpighian cells have disappeared, but the wart cells are still present. Keratinization is marked. $\times 300$.

FIG. 12. Basophilic intranuclear inclusions which occur in a few of the large wart cells. These have been observed only in older warts. $\times 1250$.



II



I2

TULAREMIC ENCEPHALITIS *

PATHOLOGY OF ACUTE TULAREMIA WITH BRAIN INVOLVEMENT AND COEXISTING TUBERCULOSIS

F. W. HARTMAN, M.D.

(From the Department of Laboratories, Henry Ford Hospital, Detroit, Mich.)

Since Francis¹ accumulated the reports of twenty-four deaths from tularemia in 1928, at least five additional reports of death have been published. Of these five cases autopsies were done in two instances, bringing the total number of reported autopsies, to date, to ten. An additional case, with two unique features and in which an autopsy was performed, is the basis of this report. The unique features are, first, involvement of the brain without involvement of the meninges, and second, the presence of active tuberculosis of the left kidney and right epididymis.

REPORT OF CASE †

Clinical History: Patient admitted December 22, 1930 at 11.30 P.M., with a complaint of fever and delirium. He had had measles and influenza, but there was no history of tuberculosis. His habits were good. Family history unimportant. On December 10, while working in a butcher shop, he lacerated his hand at the base of the left thumb. He was cleaning fish at the time of the accident, but just previous to this had been skinning rabbits. He covered the wound with iodine and continued his work. December 14, the hand became swollen and the axillary glands enlarged and quite painful. At this time he felt "feverish" and consulted his physician, who told him the injury had become infected and treated him accordingly. For three days there was local and general improvement, but December 18 more fever and malaise developed, with delirium and profuse sweats which lasted for four days. The temperature ranged from 101 to 103° F. His physician noted stiffness of neck.

Physical examination showed a well nourished and well developed adult male breathing loudly but regularly, and aroused with difficulty. The pupils were dilated and reacted to light and accommodation. The lips were cracked, dry and bled easily; the mucous membranes were coated. Throat clear. Teeth in good repair. Definite rigidity of neck was present. Lymph glands and thyroid of

* Read before the American Association of Pathologists and Bacteriologists at Cleveland, Ohio, April 2, 1931.

† The history and clinical course are briefly abstracted, as they will be given in a detailed report from the Department of Medicine at a later date.

usual size. Chest expansion normal, resonant throughout, respirations wheezing with few dry râles. Heart sounds regular, no murmurs. Blood pressure 118/80. The abdomen was on a level with the chest and there was no muscle spasm or tumor masses. The extremities were not unusual except an ulcer 2.5 cm. in diameter on the left thenar eminence. There was no visible lymphangitis but the axillary glands were enlarged. (Physician stated lymphangitis could be readily seen and that one axillary gland had been the "size of a lemon.") Kernig and Babinski signs negative. Hemoglobin 14.7 gm., red blood cells 4,830,000, white blood cells 11,500, polymorphonuclear leucocytes 84, small mononuclears 14, transitional cells 2. Urine: amber, specific gravity 1.018, acid, albumin 1 plus, sugar negative, no red blood cells, white blood cells or casts. Blood culture negative at thirty-six hours.

Progress: On December 29 the patient was drowsy and stuporous, the temperature 101.2° F. Many râles, especially at left base, were noted. The wound was clean and granulating. Blood culture agglutinated *B. tularensis* 1:640. December 31, red blood cells were 4,790,000, hemoglobin 13.9 gm., white blood cells 11,400. Urine, albumin 2 plus. Agglutination with *B. tularensis* 1:1280. January 1, 500 cc. of blood from a convalescent patient with a titer of 1:640 was given to the patient. January 2 the temperature was only slightly lower, but patient was rational. Three days later a papular eruption appeared, covering the back from scapulae to buttocks. The next day, January 6, he had a slight convulsion. On January 9 a spinal puncture was made which showed bloody fluid, negative culture, sugar 42 mg., white blood cells 145. January 20 the temperature was 104° F, râles were noted over right base and impairment over left base. The respirations became irregular and death took place.

AUTOPSY REPORT

Body: Middle-aged, white male 163 cm. in length. Skeleton slight, poorly nourished. Axillary glands on left enlarged. At base of left thumb there is healing ulcer 3 by 1.5 cm.

Abdomen: Surfaces smooth and glistening. Liver reaches costal margin. Spleen enlarged.

Thorax: No excess of fluid or adhesions. Pericardial sac contains 100 cc. clear fluid.

Heart: Weight 320 gm. Subepicardial fat moderate in amount, vessels tortuous but not sclerotic, right auricle and ventricle dilated and filled with liquid blood, valves intact. The myocardium is pale, but of good consistency.

Lungs: Left lung weighs 600 gm., right 650 gm. There are no scars at the apices. The peribronchial lymph nodes are enlarged and show small grayish white areas of necrosis. On the right side small grayish areas of necrosis are seen beneath the pleura and scattered through the parenchyma. Sections from the peribronchial lymph nodes show small areas of necrosis and polymorphonuclear infiltra-

tion. The epithelioid reaction is slight and no giant cells are found. Gram-Weigert and Ziehl-Neelsen stains show no bacteria. Sections from lungs show necrosis with little epithelioid or other cellular reaction and no giant cells. Gram-Weigert and Ziehl-Neelsen stains negative for bacteria.

Spleen: Weight 300 gm. The capsule is bluish red in color and shows no wrinkling, but there are a few grayish areas of necrosis shining through. On section the pulp is very soft and bloody. The necrotic areas cannot be made out. On microscopic examination the areas of necrosis are small and difficult to find. They are sharply outlined and there is some epithelioid reaction at the periphery. No giant cells are found. Stains for bacteria are negative.

Liver: Weight 1900 gm. The capsule is smooth and a few small grayish yellow areas of necrosis are seen shining through. On the dorsal surface, at the junction of the right and left lobes, is a soft necrotic area 8 mm. in diameter filled with grayish homogeneous material. The parenchyma is brick red in color and the cut surface is finely granular. Lobulation is distinct and there is no increase in fibrous tissue. Microscopic examination shows minute areas of necrosis infiltrated by leucocytes. There is no epithelioid reaction and no giant cells. Gram-Weigert and Ziehl-Neelsen stains reveal no bacteria (Fig. 1).

Kidneys: Weight, right 200 gm., left 200 gm. The capsules strip readily, leaving smooth, unscarred, cortical surfaces. On section the cortex ranges from 7 to 10 mm. and the usual architecture is well made out. In the upper pole of the left kidney the calyces are lined by grayish granulation tissue and are enlarged. Microscopic sections through involved calyces show whorls of epithelioid cells with giant cells forming typical tubercles. Acid-fast bacilli are readily demonstrated (Fig. 2).

Testicles: The left testicle is missing. The right epididymis is enlarged and indurated with a caseous abscess 1 cm. in diameter. Microscopic sections show whorls of epithelioid cells with giant cells and necrosis forming single and conglomerate tubercles. As many as ten acid-fast organisms to a single field are demonstrated by Ziehl-Neelsen stains.

Brain: Dura is of usual thickness and translucent. The convolutions are broad and flat and the sulci are correspondingly narrow. The inner meninges show no edema or infiltration. On section

through the fixed specimen the corpus callosum, basal nuclei, pons and the adjacent tissue show soft, grayish yellow, necrotic or hemorrhagic areas ranging from 0.5 to 3 mm. in diameter (Fig. 3). Microscopic sections taken through the brain and necrotic lesions in various places show a necrosis similar to that seen in glands, lungs, spleen and liver, except that it is much more extensive and that there is hemorrhage in places. There is more leucocytic infiltration also. The blood vessels within and near the necrotic areas show marked proliferation of the lining endothelium with narrowing, and in some cases, obliteration of the lumen. Gram-Weigert and Ziehl-Neelsen stains fail to reveal bacteria (Fig. 4).

DISCUSSION

With active tuberculosis demonstrated in the left kidney and the right epididymis by finding the tubercle bacillus in the lesions and by reproducing the disease in guinea pigs, the question naturally arises as to whether all the pathology and particularly that in the brain could be explained on the same basis. Aside from the clinical history which is typical of tularemia, and the high agglutination titer (1:2480) of the patient's serum for *B. tularensis*, the lesions in the lymph nodes, spleen, lungs, liver and brain are quite different from those in the left kidney and epididymis, in that the former show more necrosis, less epithelioid reaction and no giant cell reaction. Furthermore, sections from lymph glands, spleen, lungs, liver and brain were stained by the Ziehl-Neelsen method at the same time as sections from the kidney and epididymis, but no acid-fast bacilli were found in the former, while as many as ten acid-fast bacilli were found in a single oil immersion field in the latter.

Brain involvement has not previously been reported in man. However, Francis and Callender² record a negro with swelling and suppuration of the axillary glands who died after several days of stupor and coma. The clinical diagnosis was tuberculous meningitis. The autopsy showed bronchopneumonia and the spleen, liver and meninges showed opaque firm nodules. Although the pathology was interpreted at the time as tuberculous meningitis, tubercle bacilli were not demonstrated, so the possibility of tularemia was suggested later.

Experimentally Dwijkoff³ has produced hemorrhagic lesions of the brain stem in guinea pigs with *B. tularensis*.

On the other hand, it is doubtful if tuberculosis ever produces a general inflammation of the brain substances as seen in this case, that could properly be called encephalitis, at least in the absence of meningeal and ependymal lesions. Roque, DeChaume and Ravault⁴ report a single case of "tuberculous meningo-encephalitis," and describe the principal lesions in the meninges and ependyma with involvement of only the brain substance immediately adjacent.

In addition to lethargic encephalitis, encephalitis is known to occur in measles, scarlet fever, whooping cough, mumps, typhoid fever, typhus fever, variola, varicella and diphtheria. In tularemic encephalitis, if judged by this single observation, the lesions are even more extensive than in most of the diseases mentioned and are characterized by proliferation of the endothelium of the blood vessels, endarteritis, extensive necrosis, particularly of the white matter, and hemorrhage.

SUMMARY

1. A case of acute tularemia with diffuse encephalitis and co-existing tuberculosis of the kidney and epididymis is described.
2. The brain lesions are readily seen as areas of yellow necrosis and hemorrhage throughout the base.
3. Microscopically there is patchy necrosis with round, wandering cell and polymorphonuclear infiltration.

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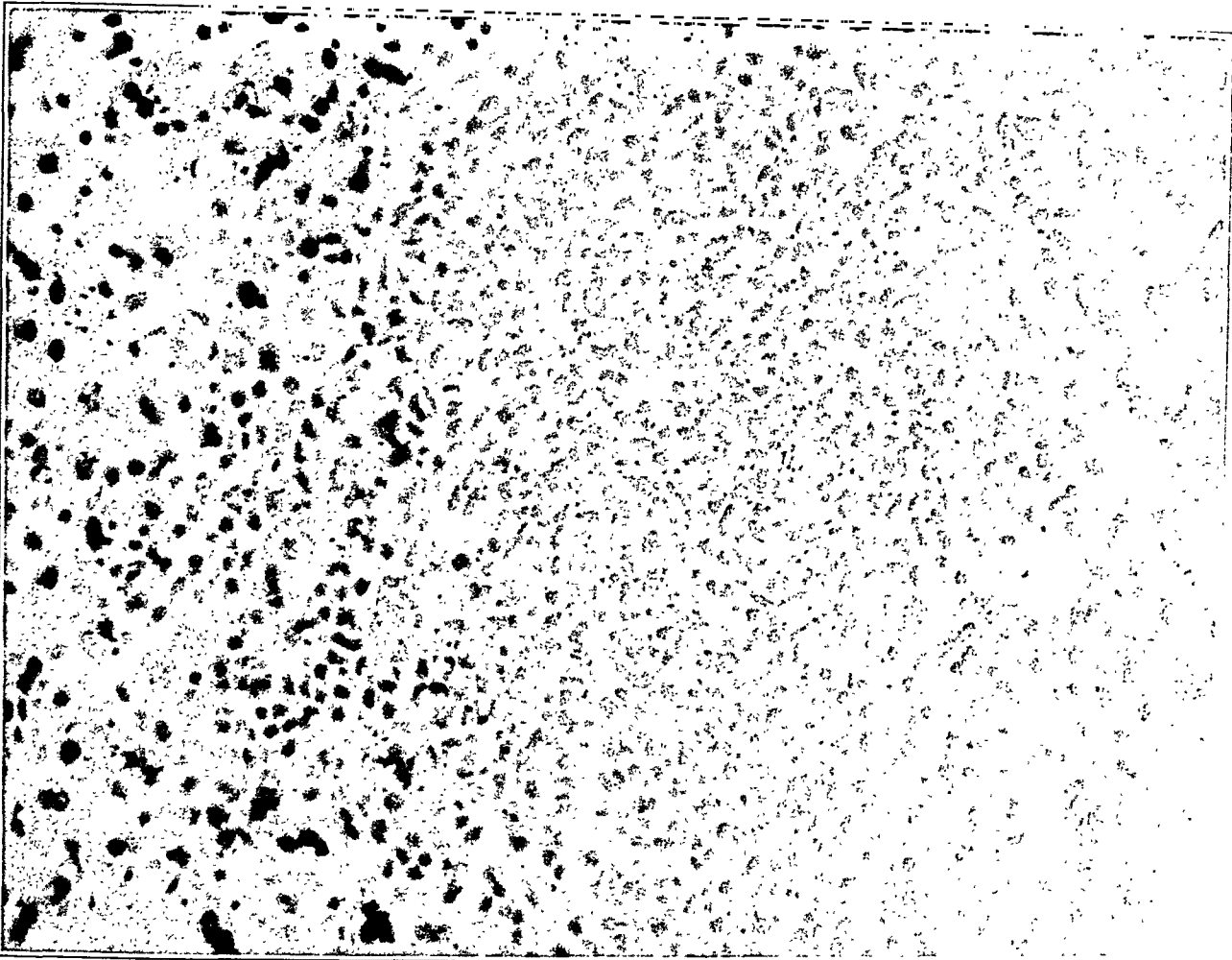
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DESCRIPTION OF PLATES

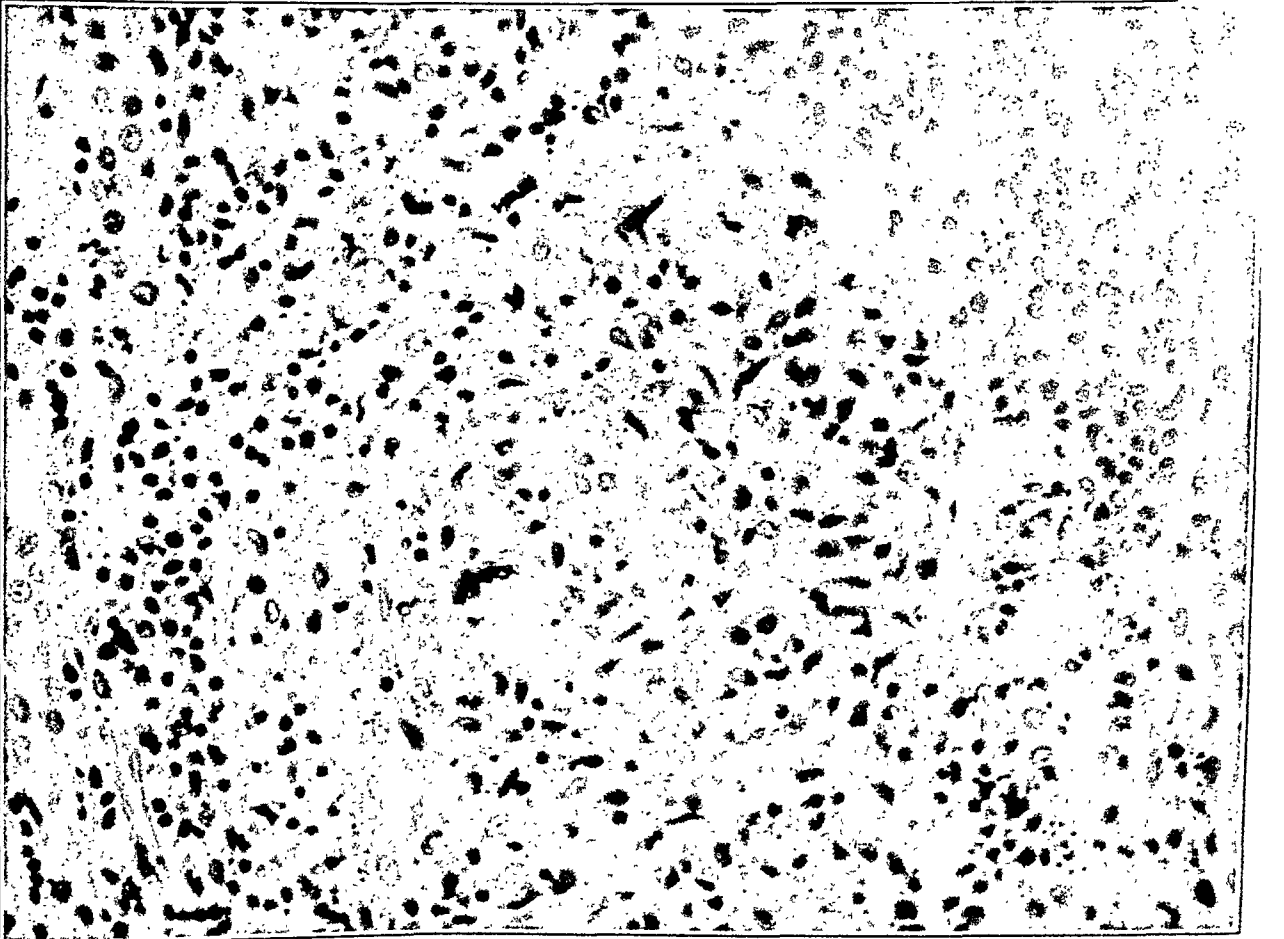
PLATE 8

FIG. 1. Photomicrograph of liver showing area of necrosis without giant cells.

FIG. 2. Photomicrograph of left kidney showing typical tubercle.



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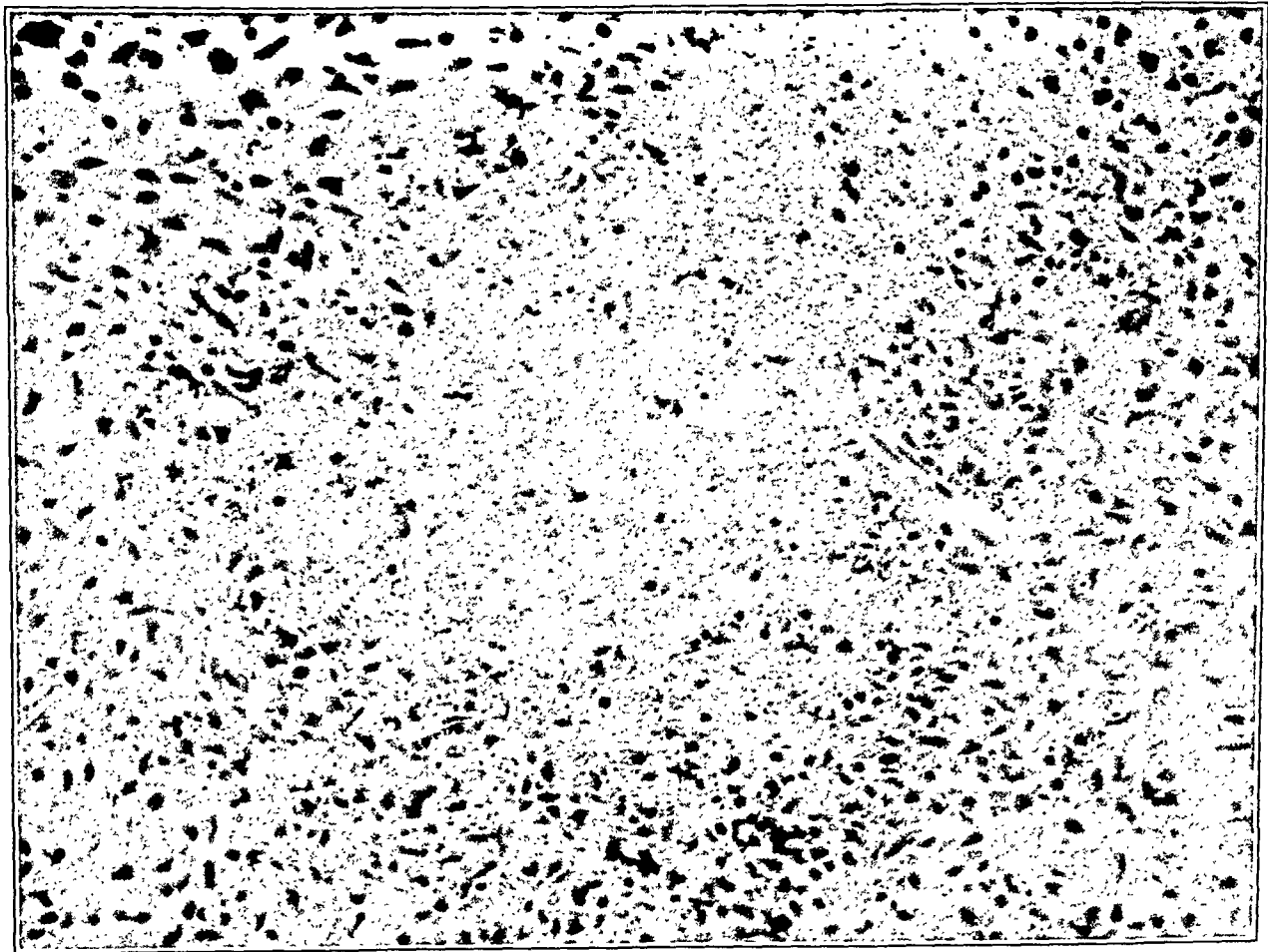
PLATE 9

FIG. 3. Coronal sections of brain showing areas of necrosis and hemorrhage.

FIG. 4. Photomicrograph of brain showing area of necrosis with leucocytic infiltration at periphery.



3



4

Hartman

Tularemic Encephalitis

A STUDY OF VACCINE VIRUS PNEUMONIA IN RABBITS *

R. S. MUCKENFUSS, M.D., H. A. MCCORDOCK, M.D., AND J. S. HARTER, M.D.

(From the Department of Internal Medicine and the Department of Pathology of Washington University School of Medicine, St. Louis, Mo.)

The results of the inoculation of vaccine virus into the lungs of rabbits have been variable in the hands of different workers. Calmette and Guérin ¹ in 1901 introduced virus into the trachea, lungs and pleura of rabbits without producing apparent lesions, although animals so treated were subsequently shown to be immune to vaccinia. Shortly thereafter Bosc ^{2,3} reported alterations in the lungs of rabbits following the introduction of vaccine virus, and in 1904 published his findings in detail. Positive results were observed in three out of twelve animals inoculated. The lungs were described as enlarged, gray or somewhat violaceous, and of the consistency of liver. Histologically, a cellular proliferation was the most prominent feature, and he spoke of the lesions as neoplastic. Endothelial proliferation in the capillaries of the involved areas was mentioned. Inclusions were observed in all of the vaccinal lesions, although no such structures in the lung lesions were illustrated.

Haaland, ⁴ in 1905, injected vaccine lymph intratracheally and observed lesions in the tracheal mucosa, usually at the site of the needle puncture, characterized by cellular proliferation and the presence of Guarnieri bodies. Lung lesions were observed, which he concluded were not caused by vaccine virus: (1) because the same lesions could be produced in vaccine-immune animals and by non-specific material; (2) the virus could not be demonstrated in the involved lung; and (3) filtrates of these lungs did not cause distinctive lesions and did not confer immunity to the virus. Numerous bodies were present in the lungs but they varied in size and had no definite relation to the nucleus, and could not be identified as Guarnieri bodies. He found no evidence of the neoplastic changes described by Bosc.

In 1929 Cattaneo ⁵ introduced neurovirus into the trachea and into the parenchyma of the lungs, producing alterations in which he

* Received for publication August 17, 1931.

discerned three stages of fibrinous pneumonia. He described numerous granules in the involved areas and thought them derived from degenerated leucocytes. He interpreted the lesion as unquestionably due to vaccine virus, since cultures of the lung tissue remained sterile and the triturated lung tissue yielded lesions typical of vaccinia when inoculated into the cornea of rabbits.

In repeating this work, but using commercial vaccine prepared in Pavia, no lung lesions were produced, although the inoculated animals became immune. This difference in the activity of different viruses may explain the discrepancies reported.

Armstrong and Lillie⁶ in 1929, using a heat-selected strain of virus, reported the production of serofibrinous pneumonia in rabbits after intratracheal or intranasal inoculation. This virus was passed from lung to lung eight times with no decrease in activity. Only one of eight immune animals developed pneumonia. Cytoplasmic inclusions were described in the bronchial and alveolar cells, as well as proliferative reactions in the blood vessels of the involved area. In a more comprehensive report published later, these findings were illustrated.⁷

The publications quoted seem to establish the fact that it is possible to cause pneumonia in rabbits by means of some strains of vaccine virus. There seems, however, to be no agreement concerning the occurrence of vaccine bodies in the reported lesions, or the rôle played by the material associated with the virus in producing the changes described in the lungs.

METHODS AND MATERIALS

Neurovirus of Levaditi, furnished by Dr. T. M. Rivers, was used. Rabbits were killed on the fourth day after intratesticular inoculation and the testicles removed aseptically and triturated with sterile sand. About 10 cc. of Locke's solution was added and the suspension centrifuged at high speed for twenty minutes. The supernatant fluid was diluted 1:10 in sterile Locke's solution before inoculation.

Animals were anesthetized and a needle was inserted through the anterior surface of the neck into the trachea, and 1 to 2 cc. of virus injected. The head of the animal was elevated so that the fluid would flow into the lungs. In some experiments a catheter was passed through the mouth and into the trachea, but as this method

was less certain the method first described was used in most of the work.

Each series of animals included some rabbits that had been immunized by skin inoculation from one to three months previously.

A total of thirty-one animals, of which thirteen were immune, was used. Three animals were inoculated with emulsions of normal rabbit testicle. Virus heated to 56° C. for three hours, virus admixed with an equal quantity of immune serum, and herpes virus (H. F. strain) were also used as controls. Virus was applied directly to a scarified area in the trachea of one normal animal.

Daily observations were made on the inoculated animals. Temperature elevations were unusual and not related to the findings at autopsy. Dyspnea was rarely observed, even shortly before the death of the animal.

The animals were killed at intervals varying from one to five days after inoculation and the lungs fixed by injecting Zenker's fluid into the trachea under low pressure. Other organs were examined, but as no changes were observed, either grossly or microscopically, they will not be described in this report.

GROSS LESIONS

Non-immune animals, in which the virus was introduced through a hypodermic needle, showed slight edema of the neck about the site of the injection. A severe form of this reaction was encountered a few times when some virus was lost in the subcutaneous tissue, because the needle did not slip into the trachea at the first puncture. These animals showed a massive edematous swelling of the loose tissue of the neck, and occasionally also of the mediastinum.

The mucosa of the trachea was always red and swollen about the needle puncture wound, and shreds of fibrin often were attached to the epithelium. Whenever lesions were found in the lungs, the mucosa of the primary bronchi and lower part of the trachea also was inflamed. If a catheter had been used there was no edema of the neck.

Gross lesions were found in the lungs of normal animals twenty-four hours after the introduction of the virus. Slightly elevated patches of translucent gelatinous consolidation and of soft doughy consistency were found usually in the posterior part of the lower

lobes. The distribution of these was lobular, but in animals with severe reactions the greater part of one lobe was involved. These patches varied in color from a grayish pink to a light brown. On section, fluid could be squeezed from the boggy areas, leaving a slightly granular surface. Tiny, yellow opaque flecks were often seen on the cut surface. Except in two discarded animals that had contracted a bacterial pneumonia, we did not encounter patches resembling the dry, gray consolidation seen in the gray hepatization stage of lobar pneumonia.

In some animals, as early as twenty-four hours, but most frequently on the second day and after, areas of hemorrhagic consolidation appeared. These seemed to represent a later stage of the edematous patches. They were sharply circumscribed, dark red areas, firm and granular on the cut surface, and resembled small infarcts. Three or four days after injection the lungs usually contained many edematous patches and a few of these dark red infarct-like areas. In one animal about half of the lower lobe showed the dark red consolidation with edematous lobules scattered throughout the remaining lung tissue.

The majority of the immune animals presented no gross lesions. A few contained small gelatinous patches and an occasional gray or red opaque fleck on the cut surface.

HISTOLOGICAL FINDINGS

Lungs: The earliest gross lesions, the areas of gelatinous consolidation, prove to be groups of alveoli filled with coagulated albuminous fluid and varying amounts of fibrin (Figs. 1 and 3). Some alveoli contain large tangled masses, others show only a few isolated strands. The cellular component of the alveolar exudate varies in different animals and in separate regions of the same lung. The primary cellular response consists of a mobilization of numbers of large mononuclear phagocytes within the alveoli and about the bronchi and blood vessels. Mitoses are numerous in these mononuclear cells. Occasionally the cytoplasm of several of these phagocytes is fused, forming a giant cell. The number of cells in different alveoli is extremely variable, ranging all the way from the few dust cells seen in normal lungs to a complete filling of the air space, with obliteration of the pulmonary markings (Figs. 4 and 8). Polymor-

phonuclear leucocytes can be found, but they do not yet form a conspicuous part of the exudate.

In many of the consolidated portions of the lung one can see necrosis of the cellular exudate and focal necrosis of the alveolar walls (Fig. 6). Here the nuclei are pyknotic or fragmented, and this degenerating and necrotic tissue is infiltrated with polymorphonuclear leucocytes. Wherever necrosis occurs, the polymorphonuclear leucocyte becomes the predominating cell. With the destruction of the alveolar walls, blood escapes into the alveoli and areas of hemorrhage appear. Widespread necrosis is always followed by a large hemorrhage producing infarct-like zones.

Blood Vessels: Characteristic changes occur in and about the larger blood vessels. The perivascular lymphatics become distended and are filled with coagulated, albuminous fluid containing many large mononuclear cells, together with a few lymphocytes and polymorphonuclear leucocytes. Later, necrosis of many of these mononuclear cells is accompanied by an increased number of polymorphonuclear leucocytes and the presence of varying amounts of fibrin. At this stage the wall of the adjacent blood vessel often shows foci of necrosis in its adventitia and even, at times, in the outer part of the media. The necrosis is either preceded or accompanied by edema of the vessel wall. We have not been able to confirm the observation of Lillie and Armstrong⁷ that the vacuoles in the media of swollen arteries are filled with a mucin-like substance, on staining with toluidin blue. However, in our material, sections stained with toluidin blue did occasionally show fine red fibrils apparently in and between the muscle fibers, but the vacuoles were always as clear as the artist has illustrated them in color (Plate 3) in the above-mentioned article. Large numbers of polymorphonuclear leucocytes invade all coats of the damaged vessel wall, and in extreme cases the intima is lifted up by a collection of inflammatory cells (Fig. 5). Thrombosis of these vessels is never seen.

Bronchi: A patch of necrotic epithelium is frequently seen in a bronchus with collections of polymorphonuclear leucocytes in and about the dead tissue. These leucocytes also infiltrate the submucosa and often extend through all the layers of the bronchial wall. A small bronchus may have its lumen occluded by a cellular exudate when the epithelium is necrotic. The larger ones are never plugged but merely have a few layers of fibrinous exudate containing leu-

cocytes, forming an elevated plaque over the strip of necrotic epithelium.

Inclusions of the Guarnieri body type were found in the bronchial epithelium in four of the non-immune animals inoculated with vaccine virus. They are round or ovoid, give the characteristic staining reactions (acidophilic), are surrounded by a clear zone in the cytoplasm and often indent the nucleus (Fig. 7). Despite extensive search Guarnieri bodies were never found in the cells lining the alveoli or even in the epithelium of the alveolar ducts. Immune or control animals never showed inclusions. A great variety of fragments can be found in the degenerating bronchial epithelium and in the cells of the alveolar walls, some of which may be misleading; but if the characteristics mentioned above are strictly observed in the identification of Guarnieri bodies, there can be no confusion.

In one normal animal, after opening the trachea, we applied virus directly to a scarified area in the epithelium. Sections from this region show a profusion of typical Guarnieri bodies in the epithelial cells of the trachea, in every way similar to those found in the bronchial epithelium of the animals with virus pneumonia, as illustrated in Fig. 7.

Immune Animals: The majority of the immune animals show few microscopic lesions characteristic of a vaccine virus pneumonia. A few lungs contain small collections of large mononuclear cells about the bronchi and blood vessels (Fig. 2). One animal, however, shows large areas of consolidation in which the alveoli are stuffed with mononuclear cells and, in places, a little fibrin. The blood vessels and bronchi display lesions similar to those seen in the non-immune animals, but there are no inclusions. A zone of edematous tissue encircles these patches of pneumonia.

Control Animals: No abnormal changes are seen in the lungs of animals injected with extracts of normal testicle. Animals inoculated with heated virus, or with virus admixed with immune serum, show slight reactions similar to those seen in the majority of the immune animals. In the animals receiving herpes virus tiny collections of mononuclear cells appear about small blood vessels and bronchi in a few places, but this reaction is much less than that seen in the immune animals injected with vaccine virus.

Except in two discarded animals with positive lung cultures, bacterial stains never revealed the presence of bacteria anywhere in the lungs, bronchi or blood vessels.

DISCUSSION

It seems justifiable to conclude that vaccine virus is capable of causing a characteristic pneumonia when introduced into the lungs of rabbits, although, apparently, this property is not common to all strains of virus. The absence or minimal intensity of the reactions in vaccine-immune animals, and the failure of emulsions of normal testicle to produce lesions justify attributing the lesions described to the action of virus. Apparently, from the work of Haaland, certain non-specific substances are capable of causing lesions of a similar type, although such lesions were not encountered in the control material included in this study. Heated virus and virus admixed with immune serum caused slight reactions that were not to be compared with those resulting from the introduction of active virus. Acidophilic cytoplasmic inclusions were observed in the bronchial epithelium of only four animals, and in none of the immune animals or control material, although it is possible that serial sections might have revealed their presence more frequently. The presence of numerous inclusions (similar to those seen in the bronchi of animals with virus pneumonia) in the tracheal epithelium to which vaccine virus had been applied directly, leaves little doubt that they are true Guarnieri bodies. On the other hand, the numerous other granules seen in cells in the involved areas of the lungs could not be identified as Guarnieri bodies.

The animals that we injected with herpes virus showed few changes; however, this virus also seems capable of producing severe pulmonary reactions. In experiments upon rabbits with the Frank strain of herpes virus, Hoffman⁸ produced a definite pneumonic process that was largely interstitial in character. The degree of the reaction varied in different animals, but some showed a massive consolidation.

In view of the changes described in the blood vessels and capillaries it might be natural to suppose that the dark red, infarct-like areas which were observed were true infarcts caused by thrombi forming in the damaged vessels. A careful study seems to show that they were not formed in this manner, but were probably due to the direct necrotizing action of the virus upon the cells of the alveolar walls in a localized area, leading to an escape of blood from damaged capillaries. Focal necrosis of cells lining the alveoli was seen before thrombi were visible in the capillaries. Furthermore, thrombi were

never present in arteries or veins even when leucocytes invaded all the coats of a vessel wall. Stewart and Duran-Reynals⁹ hold a similar mechanism responsible for the focal liver lesions found in generalized vaccinia.

The fact that Bosc described neoplastic lesions becomes intelligible, when one encounters the enormous number of large mononuclear cells that occasionally are seen in a lung, completely obliterating the pulmonary alveoli, as illustrated in Fig. 3.

It must be pointed out that the vaccine virus pneumonia is unlike the ordinary bacterial pneumonia commonly seen in rabbits. In the former the areas of consolidation are lobular in distribution and not diffuse. The cells of the alveolar exudate are exclusively large mononuclears, until necrosis takes place, when polymorphonuclear leucocytes appear. Plugs of polynuclear cells are never seen in the bronchi and seldom in the alveoli, and, of course, bacteria are never present. The lesions seen in the blood vessels are not found in bacterial pneumonia; and finally, Guarnieri bodies are present in the epithelial cells of the bronchi in a certain percentage of animals, although they are not necessary for the recognition of the virus pneumonia.

SUMMARY

1. A characteristic form of pneumonia can be produced in rabbits by the introduction of vaccine virus into the lungs. The alveoli first contain coagulated albuminous fluid and fibrin, and later a cellular exudate composed principally of large mononuclear cells. Necrosis of the exudate and of the alveolar walls leads to hemorrhage and to the appearance of polymorphonuclear leucocytes.

2. The perivascular lymphatics are distended with coagulated fluid. The walls of many of the larger blood vessels are edematous and often show a diffuse infiltration of all the coats with polymorphonuclear leucocytes.

3. Guarnieri bodies have been demonstrated in the epithelial cells of the bronchi in four animals.

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DESCRIPTION OF PLATES

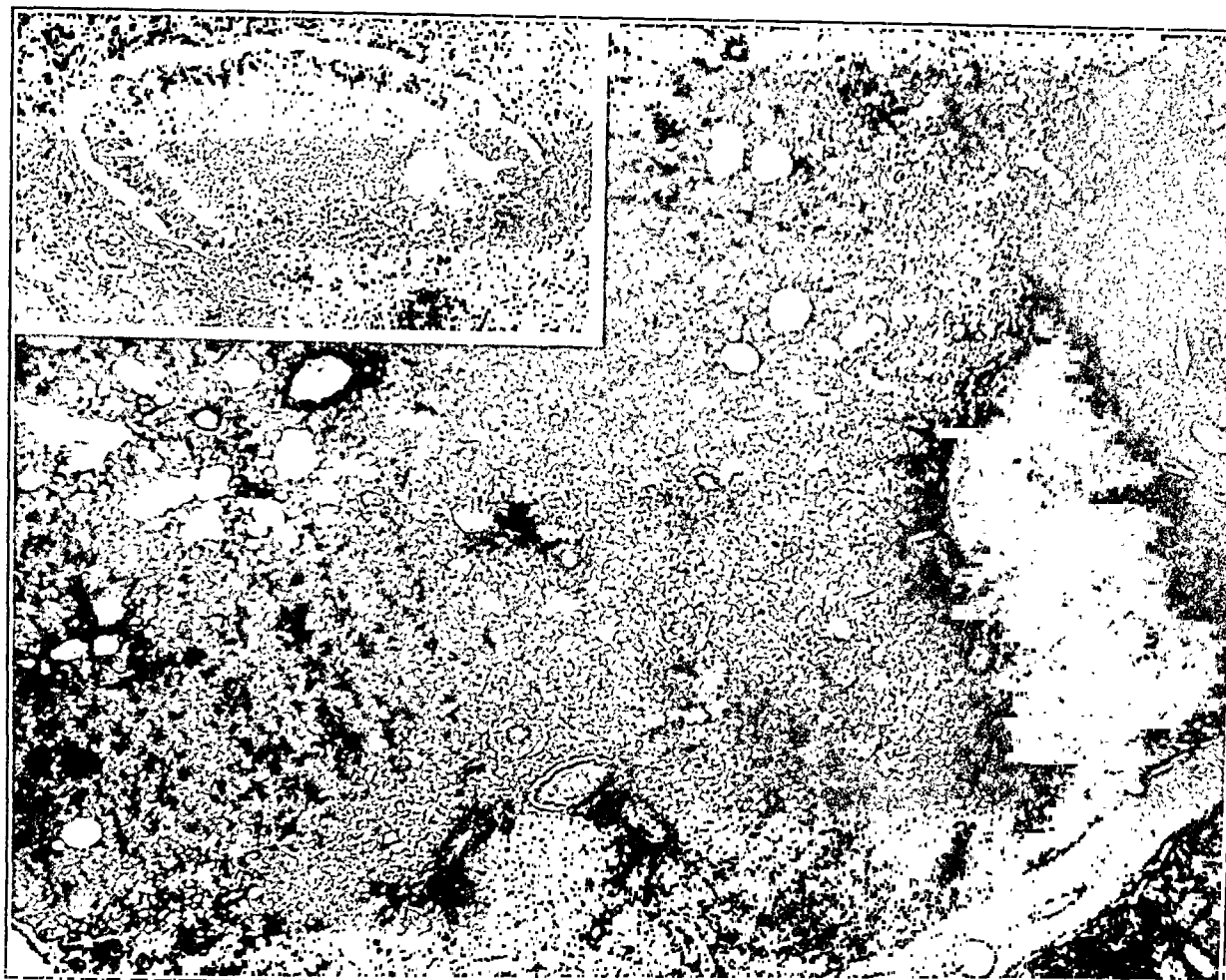
PLATE 10

FIG. 1. Virus pneumonia in normal rabbit forty-eight hours after inoculation. The black zone at the right is the infarct-like lesion produced by necrosis and hemorrhage, while coagulated edematous fluid fills most of the remaining alveoli. A scant cellular exudate can be seen in the light zone contiguous to the necrotic tissue. $\times 25$.

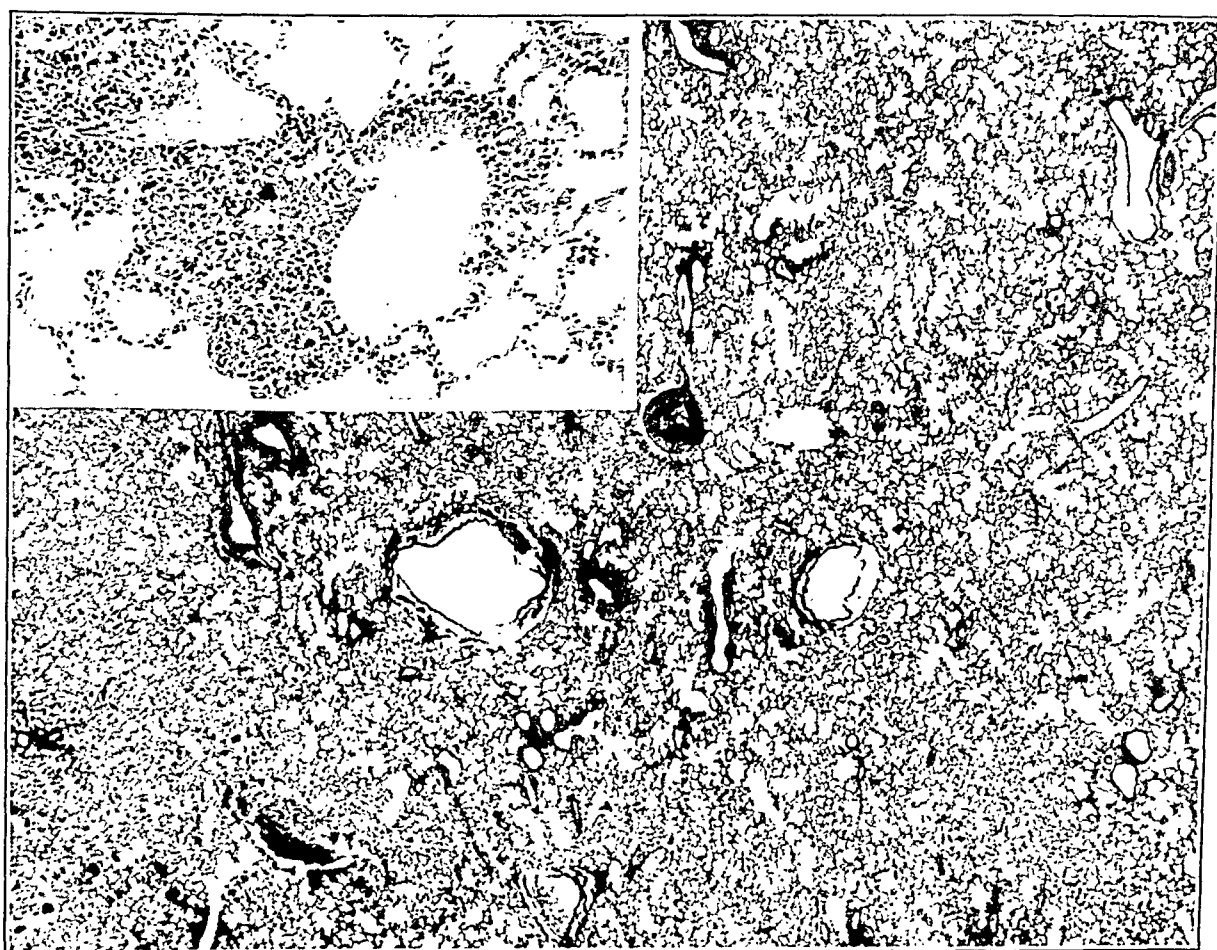
The insert is a higher magnification of the bronchus seen in the lower central portion of the low power field, and shows necrosis of a strip of epithelium, a lymph follicle and adjacent lung tissue. $\times 100$.

FIG. 2. Reaction in lung of vaccine-immune animal forty-eight hours after inoculation. Small foci of consolidation can be seen scattered throughout the section. These are mostly about bronchi and blood vessels. Note the absence of edema and necrosis. $\times 25$.

The higher magnification of one of these foci shows most of the cells to be large mononuclears. $\times 100$.



I



2

In 50 cases of nephritis he found one or more enlarged parathyroid glands. Other authors, including MacCallum, have reported the coincidence of parathyroid enlargement with nephritis. Hoffheinz ¹⁷ in 1925 collected 45 cases of parathyroid tumors; in 17 of these there was an associated generalized osteitis fibrosa cystica, 8 cases with osteomalacia, and 27 with various other bone diseases.

The concept of the relation between tumors of the parathyroid gland and generalized fibrocystic disease has been changing within the last few years.

Erdheim in 1911 ¹⁸ put forth the hypothesis that the enlargement of the gland was a compensatory effort to assist in replacing lost calcium to the bones. This theory held sway for many years.

In 1923 Dawson and Struthers ¹⁹ stated that the glandular hyperplasia was an effort to prevent the excessive excretion of lime salts and also control and prevent the development of an excess amount of guanidine, which was considered toxic. The first intimation that hyperparathyroid function might be a cause of generalized fibrocystic disease of bones through withdrawal of calcium was in 1915, when Schlagenhauser ²⁰ advised parathyroidectomy in two cases of generalized fibrocystic disease associated with a parathyroid tumor: Maresch favored the procedure but Bauer rejected the proposal. In 1925 Mandl ²¹ transplanted parathyroid tissue into a patient with generalized fibrocystic disease and the patient's condition became worse. He then removed the transplanted parathyroid tissue and a parathyroid tumor, and there was improvement of the fibrocystic disease. Gold ²² performed a parathyroidectomy in 1927, with similar improvement of the patient's bone disease.

Barr and his coworkers ²³ had a parallel case in 1929. Since this time there have been reported 23 cases of generalized fibrocystic disease of bones associated with parathyroid tumor, in each of which the tumor was removed, with subsequent improvement of the bone condition.

Collip in 1925 ²⁴ described blood calcium elevation after parathormone was injected in dogs, and also an increase in blood phosphorus in parathyroidectomized rabbits.

Greenwald and Gross in 1925, ²⁵ and again in 1926, ²⁶ showed that daily parathormone injection of 100 units in animals elevated the serum calcium and caused excretion of urine calcium and phosphorus, the former to as much as six times normal. Hunter and Aub ²⁷ also

FIBROCYSTIC DISEASE OF THE BONES ASSOCIATED WITH TUMOR OF A PARATHYROID GLAND *

REPORT OF A CASE

RAYMOND S. ROSEDALE, M.D.

*(From the Pathological Laboratory of the Buffalo City Hospital, and the
University of Buffalo, Buffalo, N. Y.)*

Generalized fibrocystic disease of bone was first described as generalized osteitis fibrosa cystica by von Recklinghausen¹ in a Festschrift to Virchow in 1891. The earliest references we have to tumor of the parathyroid glands are those of De Santi² in 1900 and Benjamins³ published in 1902; bone disease was not mentioned. Erdheim⁴ in 1903, Hulst⁵ in 1904, and MacCallum⁶ in 1905, also each reported a case without associated bone disease. Askanazy⁷ reported finding a parathyroid tumor in association with osteitis deformans in 1904, and von Verebely⁸ reported a case of parathyroid tumor with bone changes in 1907. Weichselbaum⁹ described a parathyroid tumor in 1906 without associated bone changes. Erdheim¹⁰ described three cases of osteomalacia with parathyroid enlargement in 1907. Thompson and Harris¹¹ described a similar case in 1908. Seven cases of parathyroid tumor without mention of bone changes were collected and one of his own added by Da Costa¹² in 1909. Bauer¹³ in 1911 reported a case of adenoma of the parathyroid in a 45 year old woman with a moderate degree of osteomalacia. In 1913 Molineus¹⁴ described osteomalacia in three elderly females, two of whom had each one parathyroid tumor, while the third had two distinct tumors of parathyroid tissue. Harbitz¹⁵ in 1915 noted the "relationship between enlargement of the parathyroid glands, rickets and other diseases affecting the bones."

Tumors of the parathyroid gland have been reported as coincident findings in several diseases. Bergstrand¹⁶ in 1921 reported tumor of the glands in nephritis, tetany, epilepsy, eclampsia and osteomalacia.

* This case was reported in abstract before the Buffalo Pathological Society on February 19, 1932.

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ful pulsation of the neck vessels, and a fine tremor had been present for three years. A precordial pain had been interpreted at one time as pericarditis. Night and day frequency of urination was constant.

The patient was an elderly, emaciated female with drawn and haggard features. The bony prominences and hollows were marked. The skull was seemingly larger than normal in contrast with the face bones. There was a slight exophthalmos and a suggestion of nystagmus; the sclerae had an icteric tinge. The neck vessels pulsated forcibly; a tender firm nodule was palpable in the lower lateral portion of the right thyroid lobe. Most of the teeth were missing; the few remaining ones were carious. The heart was slightly enlarged to percussion, the left border measuring 8.5 cm. to left of the midline in the fifth intercostal space, and the right 3.5 cm. to the right of the midline in the fourth interspace; there were two cardiac murmurs, a mitral systolic blow and a rough aortic systolic. Extra systoles were frequent. The systolic blood pressure was 122, and the diastolic 68. No arteriosclerosis was detected. The muscles of the extremities were atrophic, their tone poor. A slightly tender area was found over the lower third of the right tibia. The calcaneal regions were tender.

Pulse and respirations were normal. The temperature occasionally rose to 99.8 and 100° F. All other points of the anamnesis were negative.

Laboratory Studies: Red blood cells 2,450,000; hemoglobin 58 per cent (Sahli); color index 1.2; white blood cells 10,100; differential normal. A series of blood calcium determinations yielded 16.5, 13.82, 14.52 mg. per 100 cc. The blood phosphorus was 2.3 mg. per 100 cc. of blood. Urine: specific gravity ranged constantly between 1006 and 1013; albumin was 1 plus to 4 plus; occasional rare granular cast. Bence-Jones protein reaction was positive on one occasion and negative on another. Basal metabolic rate was within normal limits. The X-ray revealed cystic areas in the ribs, clavicle, scapulae, humeri, tibiae and mandible, vacuolization of the skull and a generalized loss of density of all the bones.

Biopsy material from the right tibia was obtained April 18, and was diagnosed as fibrocystic disease of bone.

On May 15, 1931, a tumor mass was removed from behind the right thyroid lobe. Local anesthesia was used. Following this the patient became stuporous with nervousness, apprehension, clonic twitchings of hand and face muscles, and could be aroused only by parathyroid and calcium therapy. The serum calcium determination on the same day, after 20 cc. of 5 per cent calcium chloride and 2 cc. parathormone was injected intravenously, was found to be 9.2 mg. per 100 cc. of blood.

With the onset of dyspnea, cyanosis and vasomotor collapse, an acute urinary suppression supervened. A blood urea determination showed 68.5 mg. per 100 cc. of blood. Death followed four days after operation. Autopsy was refused.

BONE BIOPSY

The material received from biopsy* consisted of two fragments of bone each 1 cm. square and 3 mm. thick, and two bone fragments about 2 mm. in diameter, one minute piece of soft white tissue about 1 mm. in diameter, and some blood clot bulking about 1 cc.

* The bone biopsy and the parathyroid gland tumor were submitted by Dr. H. N. Kenwell and Dr. Pietro Blanco of the Buffalo City Hospital.

found the same hypercalcemia with increased calcium excretion in man in 1926.

Albright, Bauer, Ropes and Aub²⁸ have demonstrated a negative calcium balance in animals and man receiving parathormone injections. Finally, the disease picture, both gross and microscopic, of generalized fibrocystic disease of bones has been produced repeatedly by Jaffe, Bodansky and Blair,²⁹ and also by Byrom working with Hunter and Turnbull.³⁰ More recently Johnson and Wilder³¹ reported that repeated injections of parathyroid extract produced in puppies and young rats uniform bone lesions characteristic of generalized fibrocystic disease of the bones, and concluded that the disease observed in man was due to an oversupply of parathyroid hormone with consequent loss of bone calcium.

Thus the interrelationship of generalized fibrocystic disease of bone and hyperparathyroidism has gradually been established. We have found 31 reported cases of generalized fibrocystic disease of bones associated with enlargement of a parathyroid gland. Some of these were reported as hyperplasia, others as adenomas, and one as a malignant adenoma. All of these present definite clinical and laboratory data to establish further the causal relationship of these two conditions. One additional case is presented herewith.

CASE REPORT

Clinical History: D. S., age 50 years, white, female, married housewife, entered the Buffalo City Hospital April 9, 1931, complaining of muscle aches, fatigability, pain about the knee joints and lower part of the right tibia, and loss of use of the lower limbs because of weakness.

As a young girl she had had a goitrous swelling of the neck for which she was treated with iodine. Following this the swelling disappeared. At the age of 27 years she had a periapical tooth infection that left her with a condition that was termed chronic osteomyelitis; this resisted all ordinary attention, a sinus persisting until a radical surgical procedure caused it to heal after two years.

Constipation had been obstinate for thirteen years. Seven years ago she had what was interpreted as sciatica, the pain being in both gluteal regions. For the past three years a sense of soreness was present in both heels; this was aggravated by weight-bearing. She had lost 71 lbs. in weight in this three year period, the former maximum weight being 160 lbs. About three years prior to hospital admission she had had an attack of sharp, right upper quadrant pain with sudden onset and cessation, which was interpreted at the time as renal colic.

For the last two years needle-like pain was experienced around the knees; for the past five months a pain was sensed over the lower third of the right tibia. This, aggravated by motion, has been present constantly.

She had been conscious of a firm swelling in the right thyroid region, and more recently a sense of tightness. There had been no dysphagia. Nervousness, force-

As before, multinucleated cells or osteoclastomata were seen, but they were not so large as those previously described, and they had a deeply staining nucleus. They were concentrated here more on the inner (marrow) aspect of the osteoid tissue. Between the limiting membrane of the osteoclastoma and the surrounding structure there was in many instances a clear area. One received the impression that the osteoclastic activity here was about two or three times that of the osteoblastic process.

One cc. of a brownish red fluid was obtained from a cyst in the right tibia during the biopsy. Direct smears of this showed an occasional Gram-positive short bacillus, thought to be contamination. The Rivalta test was 4 plus. Cultures did not exhibit any bacterial growth. Smears of the fluid stained with hematoxylin and eosin showed many normal appearing red blood cells, about five lymphocytes per high power field, and a rare three or four-lobed polymorphonuclear neutrophilic leukocyte. In one field there was seen a faintly pinkish gray, vacuolated, roughly circular "ghost" cell, which contained darker bluish pink, indistinctly outlined structures. This looked as though it might be a degenerated osteoclast.

The tissue diagnosis was fibrocystic disease of bone.

PARATHYROID TUMOR

The nodule removed from the thyroid region was a piece of tissue that weighed 7 gm. This was irregularly elongated and somewhat V-shaped. It measured 2 cm. through its long diameter and 2.5 cm. across, and was dark brown in color. It appeared to be well encapsulated.

Sections of the tumor stained with hematoxylin and eosin exhibited a thin fibrous capsule from which bundles of connective tissue swept centripetally, septating the glandular parenchyma. A delicate fibrous reticulum supported the glandular cells. These resolved themselves into several types. There were lobules of brighter, pinker staining tissue which were found to be chiefly large polyhedral cells, with a moderate deeply staining granular cytoplasm and oval nuclei which were a light pinkish blue with few granules. The cells had an alveolar arrangement in some fields, and the alveolar spaces contained a pinker staining, amorphous material. Scattered among these pale oxyphilic cells were basophilic or principal cells.

The sections stained with hematoxylin and eosin exhibited strands of osteoblastic tissue running at irregular angles from the periphery inward, forming a totally irregular pattern with no osseous structures present. The cells of the strands had a very scanty, faintly staining cytoplasm; their nuclei tended to be round or oval, and had within them a deeply staining chromatin material within which darker granules were seen with the aid of the oil immersion objective. Some thin-walled vascular structures were found throughout the section. The above type of cells yielded gradually by transition to larger paler cells with paler staining nuclei, the cytoplasm being more abundant and of a granular nature. Adjacent to and between these latter cells a mature fibrous reticulum was noted in a background of homogeneously light grayish pink vacuolation. Blood vessels in these latter areas were scarce, but here and there in the large vacuoles an isolated red blood cell was seen.

In this and other sections multinucleated large cells were found scattered throughout the fields, but chiefly in and around the vascular, younger and more active appearing osteoblastic areas. These cells approximated 15 to 20 microns in diameter. The cytoplasm was stained a soft grayish pink and the nuclei pinkish blue; these were round and oval and situated eccentrically, and had sharp outlines. They contained mostly a nucleolus and in some cases many small dark blue granules. The nuclei approximated about 4 microns in diameter. The greatest number of nuclei counted in any one giant cell was 28; the least number was 5. The cytoplasm of the multinucleated cells was irregularly and faintly outlined and within the cytoplasm of several of them fragments of red blood cells were seen.

In another section of bone the osseous tissue had been partly replaced by osteoid tissue stained a light pink, and in some places a deeper bluish pink. The homogeneity of the osteoid tissue was broken only by irregularly situated elliptical cells in lacunae. These cells had scant cytoplasm and oval nuclei with poorly staining chromatin material.

Between the osteoid areas, and dipping into them in finger-like processes were groups of osteoblasts and fibrous tissue strands. At the periphery of the osteoid tissue the osteoblasts were larger than elsewhere, the cytoplasm was clear and the nuclei were stained a deep blue. The cells close to the areas of osteoid tissue were larger and had more deeply staining chromatin than those farther away.

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These latter had slightly larger nuclei which stained a purplish blue and contained coarse granules. In one section basophilic cells formed alveoli containing an amorphous acidophilic substance. The principal or basophilic cells were scanty in number and the cell membrane was not clearly seen. Occasionally an acidophilic cell was seen among the nests of basophilic cells.

An iron stain, using the Prussian blue reaction, showed some small granules of black material in some of the connective tissue septae.

A fat stain (scharlach R) revealed some intercellular fat globules, mostly in a region of the chief cells, but also in some of the capillaries.

Small, thin-walled capillaries were frequently seen among groups of cells. There was one field that showed cystic change with a brownish staining pigment in the cystic areas and in the surrounding connective tissue structures. No mitoses were seen. Only two types of glandular cells were noted. No evidence of irregularity of the cellular proliferation was noted.

The tissue diagnosis was adenomatous hypertrophy of a parathyroid gland, with cystic degeneration.

SUMMARY

1. The literature concerning the development of the concept of the relation of generalized fibrocystic disease of bones to hyperparathyroidism has been reviewed.

2. A case of generalized fibrocystic disease of bone in correlation with a tumor of parathyroid gland has been presented.

The author is indebted to Dr. William F. Jacobs, Pathologist-in-Chief of the Buffalo City Hospital for the use of this material, and for his criticism in its development.

DESCRIPTION OF PLATE

PLATE 118

FIG. 1. Bone biopsy. Hematoxylin and eosin stain. Hyperchromatic osteoblasts are seen at the edge of one island of osteoid tissue. Osteoclastomata appear at the periphery of another osteoid island. There is a considerable degree of fibrous tissue between the osteoid structures.

FIG. 2. Parathyroid tumor.

FIG. 3. Parathyroid tumor. Hematoxylin and eosin stain.

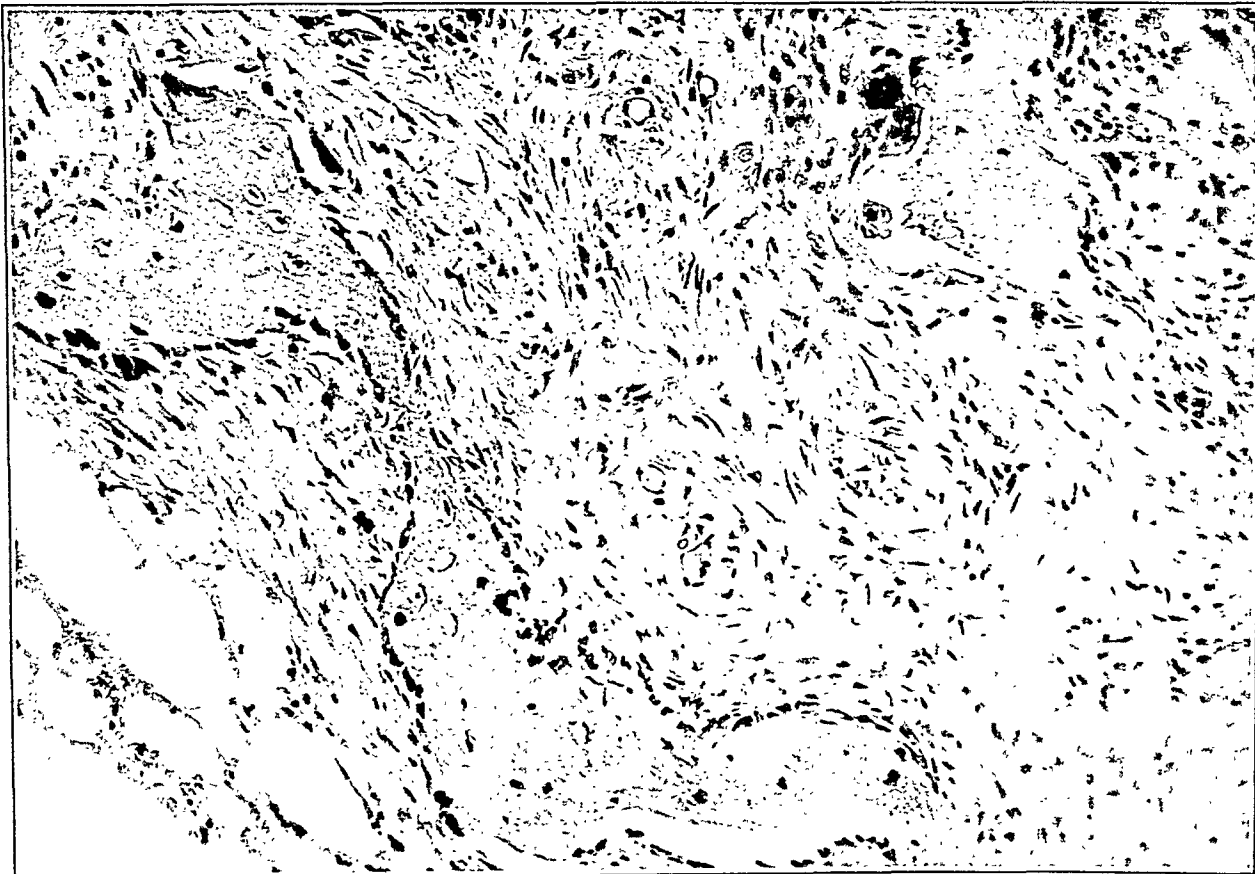
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PLATE II

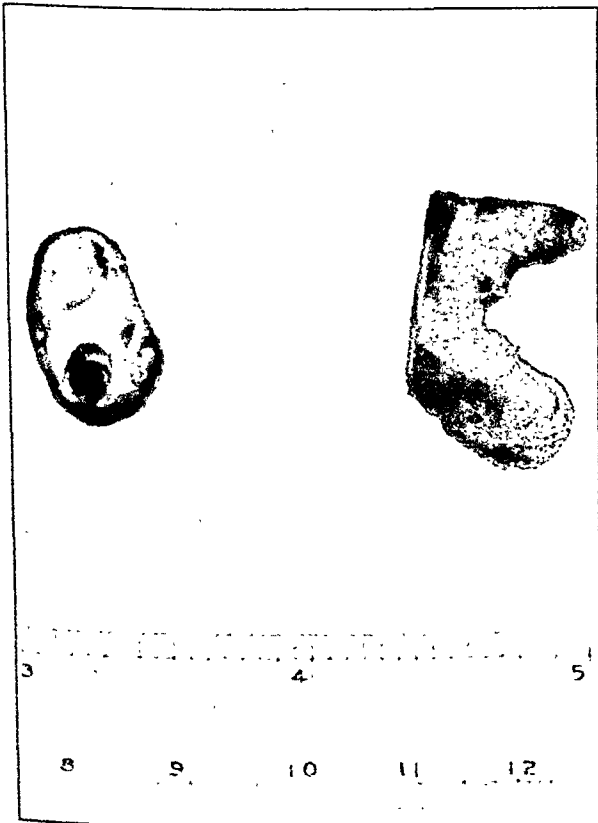
FIG. 3. Coagulated edematous fluid and fibrin filling the alveoli. Normal rabbit twenty-four hours after inoculation. $\times 150$.

FIG. 4. Cellular exudate composed principally of large mononuclear cells. Normal animal forty-eight hours after introduction of virus. $\times 400$.

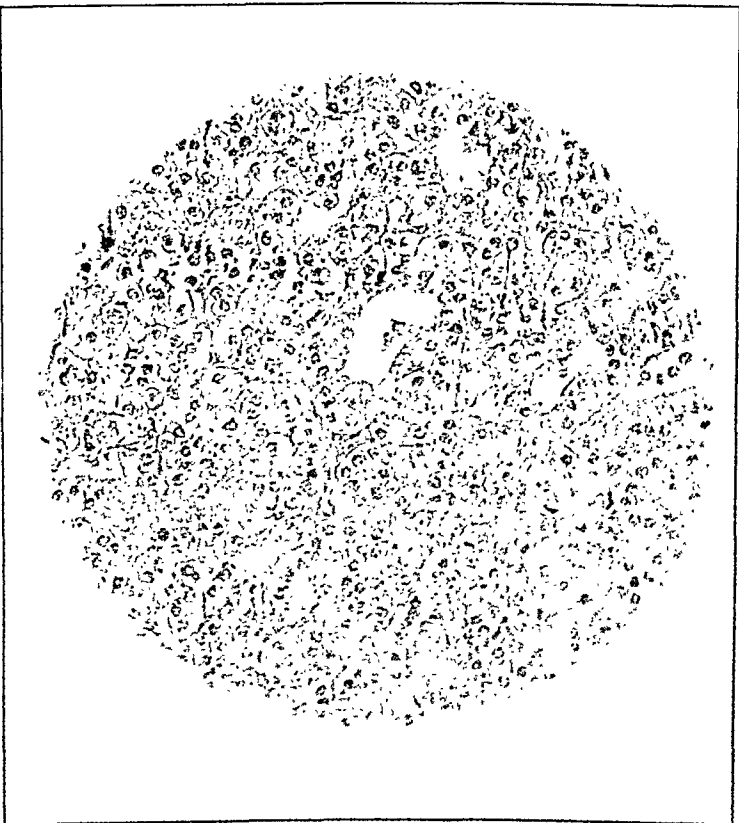
Insert shows mitotic figures in cells lining the alveoli. $\times 900$.



I



2



3

A STUDY OF THE PATHOGENICITY OF THE BACILLUS OF CALMETTE-GUÉRIN (B.C.G.) *

WILLIAM H. FELDMAN, D.V.M., M.S.

*(From the Division of Experimental Surgery and Pathology, The Mayo Foundation,
Rochester, Minn.)*

In the use of a living microorganism as a means of establishing an immune state that will be of definite protective value against spontaneous infection of a virulent bacterium, it is important that the particular microorganism used for vaccination be devoid of the ability to produce lesions of a progressive character. According to Calmette and Plotz¹ the particular bacillus of tuberculosis, known popularly as B.C.G., is definitely and permanently innocuous, although it is recommended that to ensure the perpetuity of the avirulent state the organism should be grown on glycerine-broth-potato medium with a change at certain intervals to glycerinated ox bile-potato medium, or the liquid medium of Sauton.

The contention that the avirulence of B.C.G. is of a fixed character has provided an impetus for considerable experimental work in an attempt to determine the validity of Calmette's assertions. The reports of Watson,² Petroff,³ Huttyra,⁴ Kraus,^{5, 6} Malkani,⁷ and others would indicate that under certain conditions unquestionable evidence of pathogenicity of varying degrees may occasionally occur in experimentally exposed animals. On the other hand, the results of the investigations of Remlinger and Bailly,⁸ Okell and Parish,⁹ Gerlach,¹⁰ Haring and his coworkers,¹¹ and Griffith¹² would indicate that Calmette's claim for the avirulence of B.C.G. is essentially correct.

The attempts to demonstrate the pathogenic propensities of the bacillus of Calmette-Guérin by the foregoing writers were done for the most part with cultures that were grown according to the procedure recommended by Calmette. Since, however, as pointed out by Petroff, all control of the environment of the organism is lost

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when it enters the animal body, a microörganism intended for vaccination should maintain a condition of avirulence regardless of various environmental influences to which it may be exposed. It therefore becomes exceedingly pertinent to know something of the biological behavior of B.C.G. after it is grown on culture media different from those approved by Calmette. In this regard the work of Sasano and Medlar ¹³ is of interest. Although these observers found that guinea pigs and rabbits could be inoculated with as much as 20 mg. of B.C.G. grown on bile-glycerine-potato medium without evidence of progressive tuberculosis, marked virulence was noted after their particular strain of the organism had been cultivated on a modified Sauton medium adjusted to pH 7.2 to 7.4 just prior to inoculation. Immediately before inoculation 10 per cent normal, unheated rabbit serum was added to the medium. The medium was seeded with a portion of a bile-glycerine-potato culture of B.C.G. which was procured in 1926 from Dr. W. H. Park of New York City and subcultures were made every four weeks. At the time of each subculture 2 rabbits were usually inoculated intravenously with 1 mg. of the culture. Beginning with the third subculture, 11 rabbits were inoculated to and including the eighth subculture, and marked tuberculous lesions were observed in each of the rabbits. Acid-fast bacilli were numerous in the tissue in all cases, with the disease most pronounced in the lungs. The spleen and kidneys were also affected, although lesions were not common in the liver. The infected rabbits died in from eleven to fifty days; most of them died in from twenty to thirty days after inoculation. Animal inoculations from Subcultures 1 and 2 were not mentioned. Pathogenicity of the subcultures grown on the modified medium of Sauton was also demonstrated for guinea pigs and for calves.

Sasano and Medlar wrote: "B.C.G., when grown under the environment which we succeeded in establishing, has evidenced a virulence greater than any other culture of tubercle bacillus we have had in our laboratory." They concluded that B.C.G. is not a fixed virus and that the stability of its state of virulence depends entirely on its environment.

The striking results obtained by Sasano and Medlar caused Boquet ¹⁴ of the Pasteur Institute to attempt to duplicate the experiment. The medium of Sauton was prepared according to the procedure followed by Sasano and Medlar, and three series of subcul-

tures were prepared using three different strains of B.C.G. At the time of making each subculture, guinea pigs and rabbits were inoculated, the former subcutaneously and intraperitoneally and the latter intravenously. Sixty-nine guinea pigs and 33 rabbits were inoculated with the respective subcultures, and although the dosage used was ten to fifteen times greater than was used by Sasano and Medlar, autopsy of the animals did not reveal the slightest evidence of tuberculosis. Having failed to confirm the observations of Sasano and Medlar, although the same technique was used, Boquet was of the opinion that although it is possible that some investigator in the future might succeed in modifying the pathogenicity of B.C.G., irrefutable proof of such modification has not as yet (1931) been recorded.

The remarkable divergence of the results obtained by what were presumably identical procedures makes it impossible to predict with certainty what influence, if any, the environment furnished by the medium of Sauton, as modified by Sasano and Medlar, may have on the virulence of B.C.G. It is of importance that the exact status of this question be established by further work.

Dreyer and Vollum,¹⁵ by subculturing two different strains of B.C.G. in large volumes of a simple veal broth-peptone medium, observed definite evidence of pathogenicity. One of the strains had previously been maintained on egg media for several generations and was considered completely avirulent for laboratory animals. Virulence of surprising severity for both rabbits and guinea pigs was observed when subcultures were grown in the depths of the bouillon mentioned. The resultant infection was progressive and was reinoculable. The second strain of B.C.G. investigated had been grown on potato medium prior to coming into the possession of Dreyer and Vollum. Of 56 guinea pigs inoculated with deep bouillon subcultures of the strain, definite progressive tuberculous lesions developed in 5. Although the degree of virulence in the second strain was much inferior to that observed in the first, it nevertheless exhibited a decided increase in pathogenicity after propagation in the depths of the bouillon mixture. Dreyer and Vollum were inclined to believe that the difference in virulence of the two strains was due to factors inherent to the respective microorganisms.

In January, 1930, a strain of B.C.G. (bovine origin) was obtained from Calmette. The organism was growing on glycerine-broth-

potato medium and was continued on a similar medium, prepared in my laboratory, until October, 1930. Subcultures were made every four weeks. Tests for pathogenicity were made by injecting 4 guinea pigs with a suspension prepared from the first subculture made from the original culture obtained from France. At autopsy none of the guinea pigs revealed lesions of a tuberculous nature.

After continuing the culture for nine months on glycerinated broth-potato medium, an experiment was planned to determine what effects a glycerinated egg medium might have on the virulence of the organism.

METHODS

Culture Medium: The medium used was a modified formula of that described by Miraglia and consisted of a mixture of egg yolks and 6 per cent glycerinated water.* Transfers were made about the twenty-fifth of each month and the tubes were incubated at 37° C until a luxurious growth appeared. This usually required about two weeks. After a satisfactory growth was obtained the cultures were placed in the refrigerator where they remained until suspensions were prepared for inoculation of animals.

Bacterial Growth: Throughout the period necessary to attain fifteen generations of the organism on the glycerine-egg yolk medium there was no appreciable change in the gross physical character of the culture. It consisted of numerous dry, discrete, grayish white colonies which eventually assumed a slight pinkish tinge. As growth continued those colonies in close proximity became somewhat confluent due to the uneven piling up of the bacterial masses. A diffuse, smooth or spreading type of growth was not observed.

Bacterial Suspensions: Portions of the growth were placed in a

* This medium is prepared as follows: (1) Seven medium-sized, strictly fresh eggs are washed in water and immersed 10 minutes in 80 per cent alcohol. (2) A portion of the shell at one end of the respective eggs is broken away carefully and with sterile, sharp pointed scissors the membranous sac is punctured; after discarding the egg-white, the yolk is discharged into a sterile mixing bowl. (3) To the egg yolks 100 cc. of a 6 per cent glycerine solution prepared as follows is added: glycerine 24 cc., distilled water 500 cc. sterilized in the autoclave for 15 minutes at 15 pounds pressure; the solution is autoclaved in 100 cc. portions and stored for future use. (4) The egg yolks and the glycerine solution are thoroughly mixed with a sterile egg beater and tubed in sterile apparatus. Precautions should be taken to minimize possible contamination. Sterilization is done in the Arnold sterilizer or the inspissator; the first day at 75° C until solidified, then at 85° C for 1 hour, and the second, third, and fourth days at 75° C. Before using, the medium should be incubated for 2 days at 37° C.

sterile mortar and mixed with a few drops of sterile physiological sodium chloride solution. By the use of a pestle a suspension was obtained which was purposely made rather dense. Using additional amounts of physiological sodium chloride solution, a suspension was secured comparable in density to Tube 10 of the McFarland nephelometer. This was permitted to remain for a few hours in the refrigerator to enable the larger clumps to settle to the bottom. The suspension was then ready for use.

Animal Inoculations: With the exception of the first subculture on the glycerine-egg medium, 4 guinea pigs were inoculated from the suspensions prepared from each of the respective subcultures. From the first glycerine-egg subculture 2 guinea pigs were injected, 1 subcutaneously and 1 intraperitoneally. The animals were obtained from our own breeding pens and were usually young adults. Each lot of 4 animals was injected as follows: 2 intracerebrally, each animal receiving 0.4 cc. of the bacterial suspension; 1 subcutaneously, and 1 intraperitoneally, each receiving 1 cc. of the bacterial suspension. The intracerebral injections were made according to a method described previously.¹⁶ After inoculation the guinea pigs were placed two in a cage and housed in a building apart from the structure in which animals used for experimental tuberculosis are kept. They were cared for by an attendant whose duties precluded the possibility of an inadvertent exposure to the germ of tuberculosis, and were fed the regular food provided for the other guinea pigs maintained at the institution.

The inoculated animals were observed daily and dead animals were usually examined either immediately or within a few hours after death. The inoculated animals were permitted to live for a period of from six months to one year after injection unless they died. At the time of autopsy, portions of the principal organs were placed in 10 per cent neutral formalin solution regardless of whether or not there was gross evidence of disease. Sections were prepared from the respective tissues and appropriately stained to reveal the presence of acid-fast bacteria. Attempts were also made to culture acid-fast bacteria that might have been present. Ordinarily only one tissue from each animal was used for cultural purposes. The cultural procedure followed was to treat portions of the emulsified tissue with 5 per cent oxalic acid as recommended by Corper and Uyei¹⁷ and seed the material on glycerinated egg yolk media.

Tuberculin Tests: In a moderate number of instances guinea pigs were injected intradermally with 0.01 cc. of mammalian tuberculin prepared by diluting Koch's old tuberculin with equal parts of physiological sodium chloride solution. The tuberculin tests were usually administered thirty to sixty days subsequent to the inoculations with suspension of B.C.G.

RESULTS

An analysis of data pertaining to the respective guinea pigs in this experiment disclosed that up to March 31, 1932, 43 of the animals died, 11 were killed and 4 are still living (Table I). Among the

TABLE I

Summary of Results Following the Inoculation of Guinea Pigs with B.C.G.

Intracerebral inoculation	28
Intracerebral inoculation in which tuberculous lesions were found in the brain or the spleen	9
Subcutaneous inoculation	15
Subcutaneous inoculation in which tuberculous lesions developed	1
Intraperitoneal inoculation	15
Intraperitoneal inoculation in which tuberculous lesions developed	1
Total number of animals in which tuberculous lesions were observed (approximately 19 per cent)	11
<hr/>	
Animals dying spontaneously	43
Animals killed	11
Animals still living	4
<hr/>	
Total	58

animals that died, death occurred subsequent to inoculation as follows: 8 guinea pigs one to thirty days; 11 guinea pigs thirty-one to sixty days; 7 guinea pigs sixty to ninety days; 11 guinea pigs ninety to 180 days, and 6 guinea pigs 181 days to one year. The manner of injection did not seem to have significant bearing on the time elapsing before death.

Although gross lesions of a tuberculous character were not discernible at autopsy, significant lesions were found by subsequent microscopic study in 11 of the animals (Table II); 9 of these 11 had been inoculated intracerebrally, 1 had been inoculated subcutaneously and 1 intraperitoneally. In six instances in which the bacteria had been introduced into the brain, multiple and well defined tuber-

culous lesions were present in the spleen and in the brain.* That a tuberculous infection primary in the substance of the cerebrum may subsequently cause lesions in the spleen was conclusively demon-

TABLE II

Summary of Cases in which Tuberculous Lesions Developed Following Inoculations with B.C.G. All Animals Died Spontaneously

Animal No.	Inoculation	Generation of sub-culture on glycerine-egg medium	Days before death	Distribution of lesions	Results of culture
11	Intracerebral	4th	14	Brain, liver and spleen	Brain positive
12	Intracerebral	4th	14	Brain and spleen	Brain positive
18	Intraperitoneal	5th	44	Spleen	Spleen positive
23	Intracerebral	7th	14	Brain	Brain positive
24	Intracerebral	7th	31	Brain and spleen	Brain positive
28	Intracerebral	8th	26	Brain, spleen and liver	Brain and spleen positive
51	Intracerebral	14th	38	Brain and spleen	Brain positive
52	Intracerebral	14th	32	Spleen ¹	Brain positive
55	Intracerebral	15th	52	Brain	Brain positive
56	Intracerebral	15th	74	Brain and spleen	Brain positive
57	Subcutaneous	15th	34	Spleen	Spleen positive

¹ Histological sections were prepared from only half of the brain. The other half yielded a culture of acid-fast bacilli.

strated by previous work.¹⁶ Therefore, the observation in this study of tuberculous lesions in the spleen as a consequence of a primary infection of the brain was not considered unusual.

A summary of the autopsy data, pertaining to the 54 guinea pigs that died or were killed subsequent to inoculation with the suspen-

*No lesion was considered tuberculous unless acid-fast bacilli could be demonstrated in suitably stained histological sections, or acid-fast bacilli obtained in cultures prepared from tissue containing the lesions.

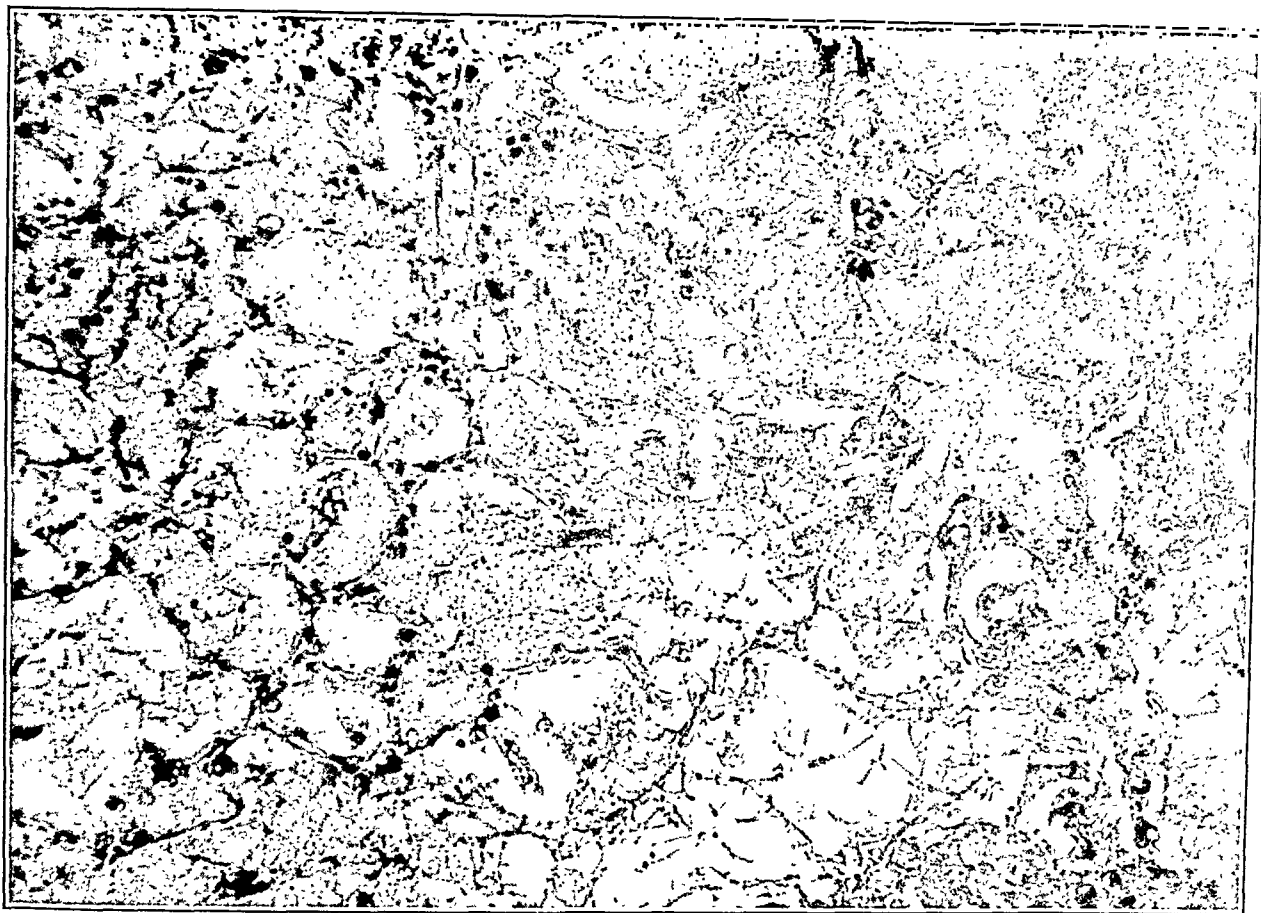
sion of B.C.G., shows that 30 of the animals were without demonstrable lesions. The major lesions noted in the remaining animals were: pneumonia, 6 animals; enteritis, 2 animals; purulent adenitis, non-tuberculous focal splenitis, hydrocephalus, peritonitis, hydrothorax, each 1 animal; and tuberculous lesions, 11 animals.

Tuberculin Tests: The attempts to demonstrate an allergic state by the intradermal injection of mammalian tuberculin were without significant results. In only a few instances were suspicious or slight positive reactions observed. Occasionally a small area of marked induration occurred, but in no instance was a typically positive reaction obtained.

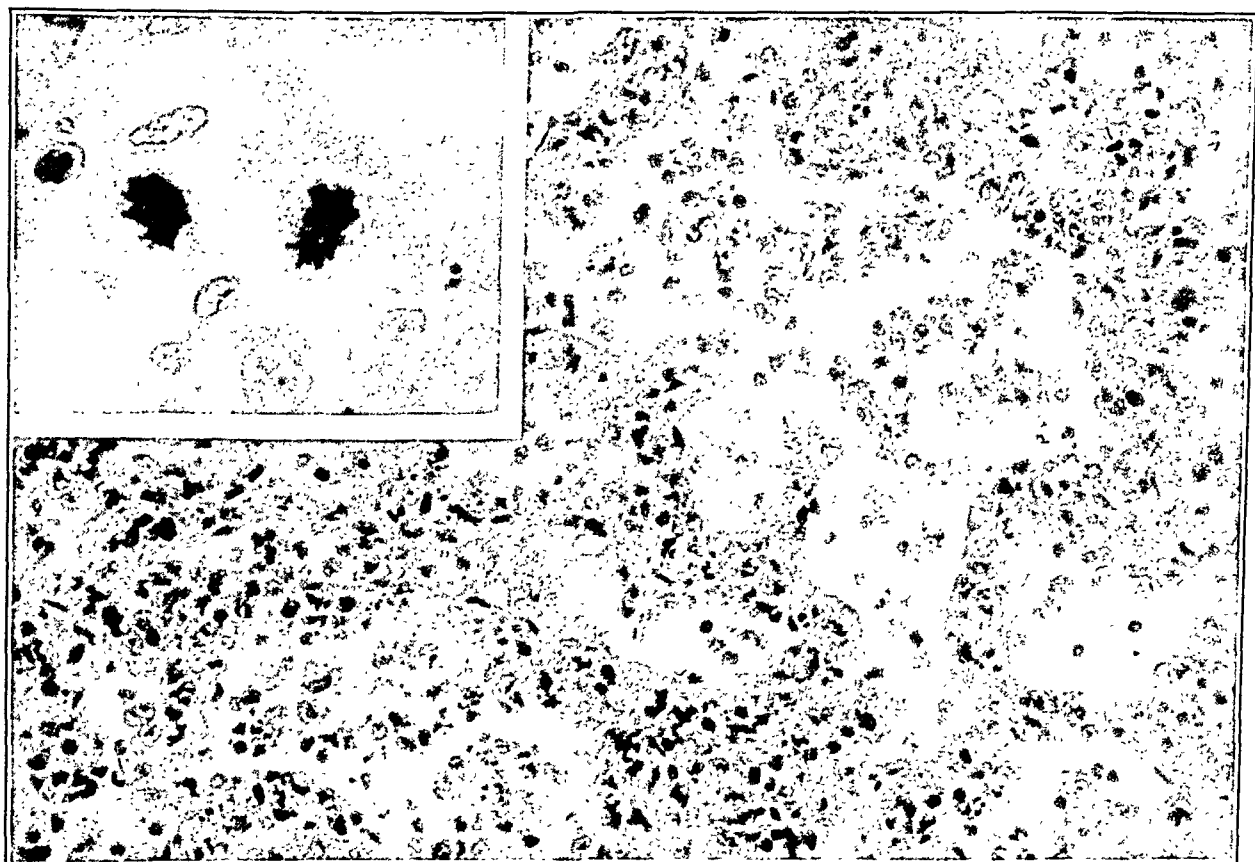
PATHOLOGICAL HISTOLOGY

The lesions of the brains of the animals that died within two weeks were usually confined to the sulci, although in a few instances there was a limited involvement of the pia mater (Fig. 1). Brains of animals in which the infection had existed for a longer period revealed lesions that extended rather diffusely into the depths of the cerebral tissue (Fig. 2). The lesions, which were composed largely of monocyctic cells, had a perivascular inception and were not circumscribed or encapsulated. Infrequently lesions were observed in which caseation necrosis was beginning. Giant cells of the Langhan's type were seldom seen, and the relatively short duration of the disease apparently seemed to have precluded the deposition of mineral salts in the necrotic areas. Acid-fast bacilli were numerous in practically all of the lesions of the brain. The monocyctic cells for the most part retained their immature characteristics and in only one instance did the cells show a tendency to assume an epithelioid appearance. This was observed in the brain of Guinea pig 55, which died fifty-two days after inoculation.

The splenic lesions were multiple. They began in the splenic corpuscles and many extended peripherally to the outer confines of these structures, replacing the lymphoid elements as a consequence. The splenic pulp was seldom violated (Fig. 3). Encapsulation was not apparent and only a minimal amount of necrosis had occurred. The cellular elements constituting the respective lesions were mainly monocyctic, but unlike those observed in the brain there was a marked tendency for many of the cells to assume an epithelioid appearance (Fig. 4). Giant cells of the Langhan's type were too infre-



3



4

One might expect the intracerebral route of inoculation to be a more vulnerable portal of entry than the intraperitoneal or subcutaneous route; therefore it seems most unusual that lesions should develop in one animal of a group after subcutaneous or intraperitoneal injection, and that lesions had not developed in one or both of the animals that received portions of the same suspension intracerebrally when examined at autopsy months later. This happened in the case of the guinea pig given injections of the suspension prepared from the fifth subculture of the organism grown on the glycerine-egg medium. Animal No. 18, injected intraperitoneally February 12, 1931, died forty-four days later with definite tuberculous lesions in the spleen from which a culture of acid-fast bacilli was obtained, yet in none of the other 3 animals in this group were lesions established that could be demonstrated either grossly or microscopically. One of the animals given intracerebral injections was living almost a year later and was finally killed for autopsy.

Individual susceptibility, rather than a general increase in the virulence of the organism, would seem the most logical explanation for the occurrence of the tuberculous changes observed in this study. The general physical condition of the respective animals in which lesions were found was not below that which obtained for cage mates that were devoid of lesions. The resistant state of the animals did not appear to be influenced by, or to be dependent on, factors pertaining to food, care or environment. In fact, the animals in which lesions occurred were generally in good, if not excellent, physical condition at the time of death, as judged by the plumpness of musculature. The reason why lesions developed in some animals and not in others cannot be explained satisfactorily.

When one considers the length of time Calmette and his collaborators cultured B.C.G. on the glycerinated bile-potato medium before the organism attained its present state of reduced virulence, it is not surprising that marked reversion did not occur after subculturing for fifteen generations on the glycerine-egg medium. It would seem logical to assume, however, that since a diminution of virulence occurred by prolonged cultivation on the virulence-reducing glycerine-ox bile-potato medium, an extended tenure on a favorable medium might bring about restoration of the initial virulent state. This hypothesis justifies a continuation of this work.

Failure to establish a progressive tuberculous infection by the re-

quent to be significant. Many acid-fast bacilli were demonstrable among the cells of the splenic lesions, although for the most part the organisms were not so numerous as in the cerebral tissues.

The lesions of both the brain and the spleen were indistinguishable from those of comparable duration in these organs following the experimental introduction of virulent mammalian strains of *Mycobacterium tuberculosis*. The histopathology was typically that of an early progressive tuberculous process with little if any evidence of a significant inhibitory mechanism.

REINOCULATION OF INFECTIVE MATERIAL

Several attempts were made to transmit the disease to other animals by emulsions of tissue of animals that had died, or by pure cultures of acid-fast bacilli obtained from the organs of animals possessing lesions of a significant character. Generous amounts of the inoculum containing enormous numbers of acid-fast bacilli were used for inoculation and in most instances rabbits, in addition to guinea pigs, were inoculated. The guinea pigs were inoculated either subcutaneously or intraperitoneally, and the rabbits intravenously. Although a few of the animals died within the first sixty days after inoculation the greater number survived to be killed at the expiration of six to eight months.* At the time of writing, none of these attempts to induce lesions of tuberculosis by injecting into animals viable acid-fast organisms obtained from lesions of guinea pigs previously inoculated with B.C.G. has been successful.

COMMENT

The data obtained from these experiments do not afford evidence that the particular B.C.G. strain used has experienced a progressive increase in pathogenicity sufficient to account for the tuberculous lesions that were found. The bacteria that incited lesions were obtained from glycerine-egg media, Subcultures Nos. 4, 5, 7, 8, 14 and 15; yet the animals inoculated with Subcultures Nos. 1, 2, 3, 6, 9, 10, 11, 12 and 13 were without demonstrable lesions of tuberculosis. It is worthy of note that not all of the animals that were inoculated manifested tuberculous lesions even though one or two may have.

* Several animals are still under observation.

SUMMARY AND CONCLUSIONS

Using a strain of B.C.G. obtained from Calmette of the Pasteur Institute, a deliberate attempt was made to increase its pathogenicity by subculturing the organism on a glycerinated egg medium. Transfers were made every thirty days. From each succeeding subculture 4 guinea pigs were given injections — 2 intracerebrally, 1 subcutaneously, and 1 intraperitoneally. The report deals with data obtained after the organism had been subcultured on glycerinated egg medium for fifteen generations.

Of a total of 58 guinea pigs inoculated, lesions histologically indistinguishable from those of genuine tuberculosis occurred in the tissues of 11, and cultures of acid-fast bacilli were obtained from each. Although the majority of the lesions occurred in animals that had been given intracerebral injections, 1 animal that was given an intraperitoneal injection and another given a subcutaneous injection died with lesions of a tuberculous nature. So far, attempts have failed to promote a succession of tuberculous lesions by the reinoculation into guinea pigs of infective material from lesions.

The particular strain of B.C.G. studied is not devoid of pathogenicity for guinea pigs, and the assertion that the organism is innocuous cannot be accepted without reservations.

Subculturing the organism on glycerinated egg medium at monthly intervals for a period of fifteen generations did not markedly enhance its virulence.

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inoculation of animals with infective material obtained from guinea pigs with definite tuberculous lesions provides an illustration which invalidates the conclusion that the virulence of the particular strain of B.C.G. used in this study has materially increased as a consequence of a prolonged residence on the glycerine-egg medium. Until it is possible consistently to promote tuberculous lesions by the reinoculation of such material, the pathogenic propensities of the organism remain problematic. On the other hand, to assert that the bacteria are entirely innocuous would hardly be acceptable on the basis of unquestionable evidence of pathogenicity in at least 11 of the guinea pigs. It is true that rather large doses of the bacterial suspensions were injected into the respective animals, but the subsequent development of tuberculous lesions in approximately 19 per cent of the guinea pigs inoculated is hardly a negligible circumstance. It seems pertinent to note also that inability to infect additional animals by the reinoculation of material from these lesions did not appreciably mitigate the severity of a disease that was characterized by the formation of definite tuberculous lesions and the eventual death of the respective animals. Whether or not it is possible to induce progressive tuberculosis that is reinoculable, the fact remains that the particular strain of B.C.G. studied, when introduced into guinea pigs in large amounts, not infrequently incited a tissue reaction of a tuberculous character that apparently resulted in death. In other words, simply because the disease was not transmissible by reinoculation did not make the lesions any less real in the respective animals in which lesions were found.

This study emphasizes the necessity of the examination of properly stained histological sections before a decision is made on the tuberculous or non-tuberculous content of a tissue. Decisions based on the gross appearance of a given tissue are frequently fallacious. In none of the tissues observed in this study could gross evidence of tuberculosis be seen, yet definite tuberculous lesions were eventually found in 11 of the animals. In work of this kind tissues should be preserved from every animal at autopsy in order that significant lesions may not be overlooked.

DESCRIPTION OF PLATES

PLATE 119

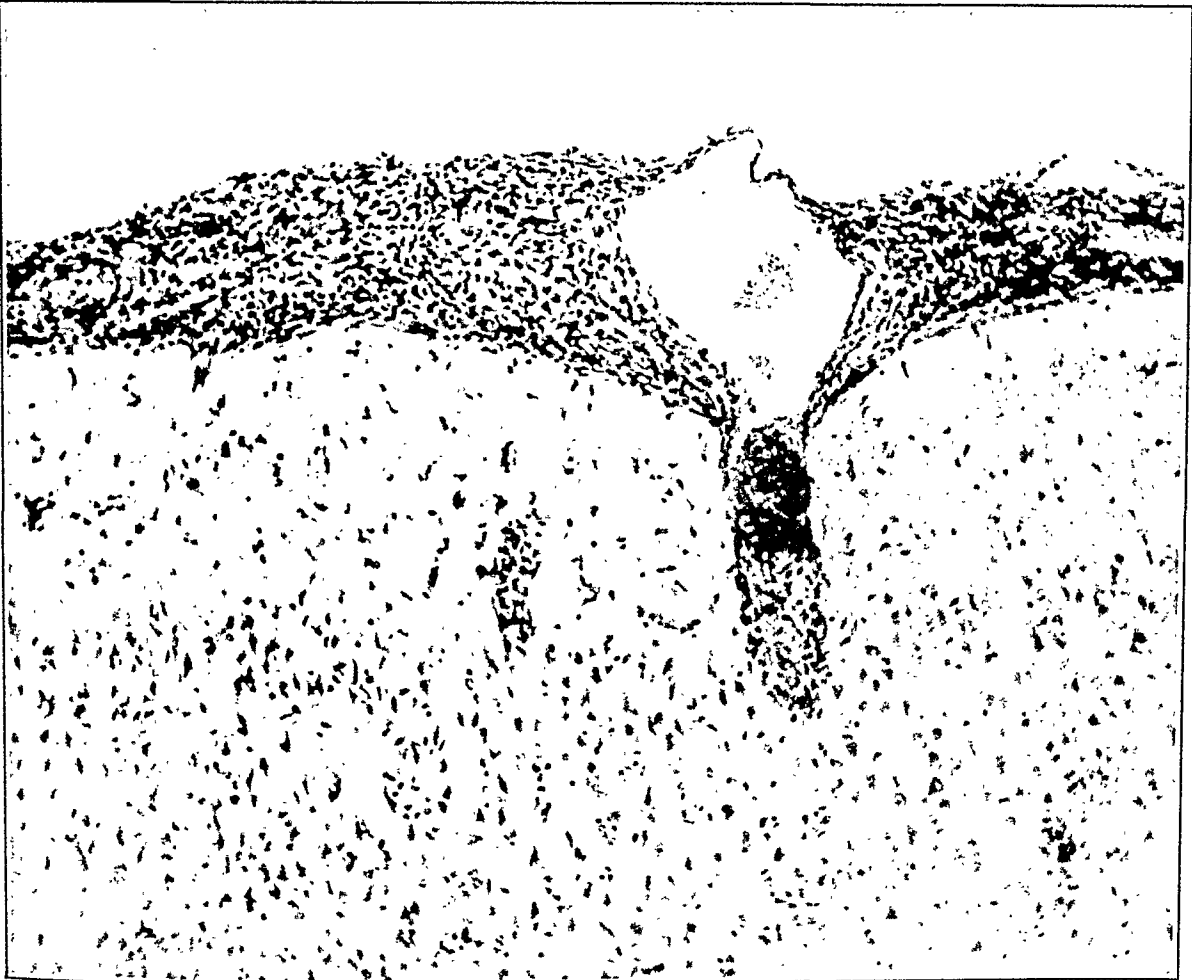
- FIG. 1. Cerebral meningitis of Guinea pig 55. Animal died fifty-two days after intracerebral injection of a suspension of B.C.G. Culture was the fifteenth generation grown on glycerinated egg medium. Lesion is essentially a monocytic reaction. Acid-fast bacilli were present in the lesion. $\times 130$.
- FIG. 2. Multiple lesions in the brain of Guinea pig 28. Animal died twenty-six days after intracerebral injection of a suspension of B.C.G. Culture was the eighth generation grown on glycerinated egg medium. Many acid-fast bacteria were present in the cerebral lesions and the spleen was also affected (see Fig. 3). $\times 140$.

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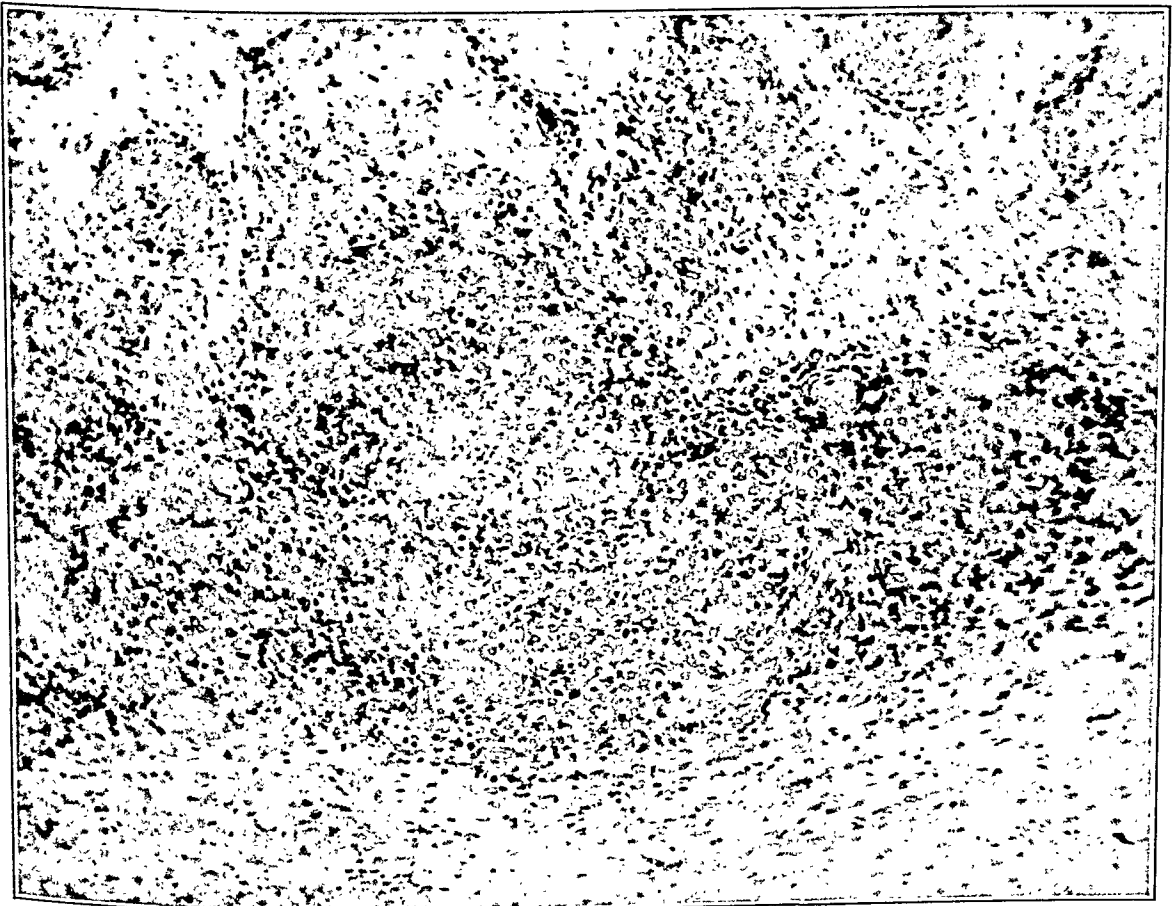
PLATE 120

FIG. 3. Spleen of Guinea pig 28. The splenic lesions were multiple and were composed largely of epithelioid cells. A culture of acid-fast bacilli was obtained from an emulsion prepared from a portion of this spleen. $\times 170$.

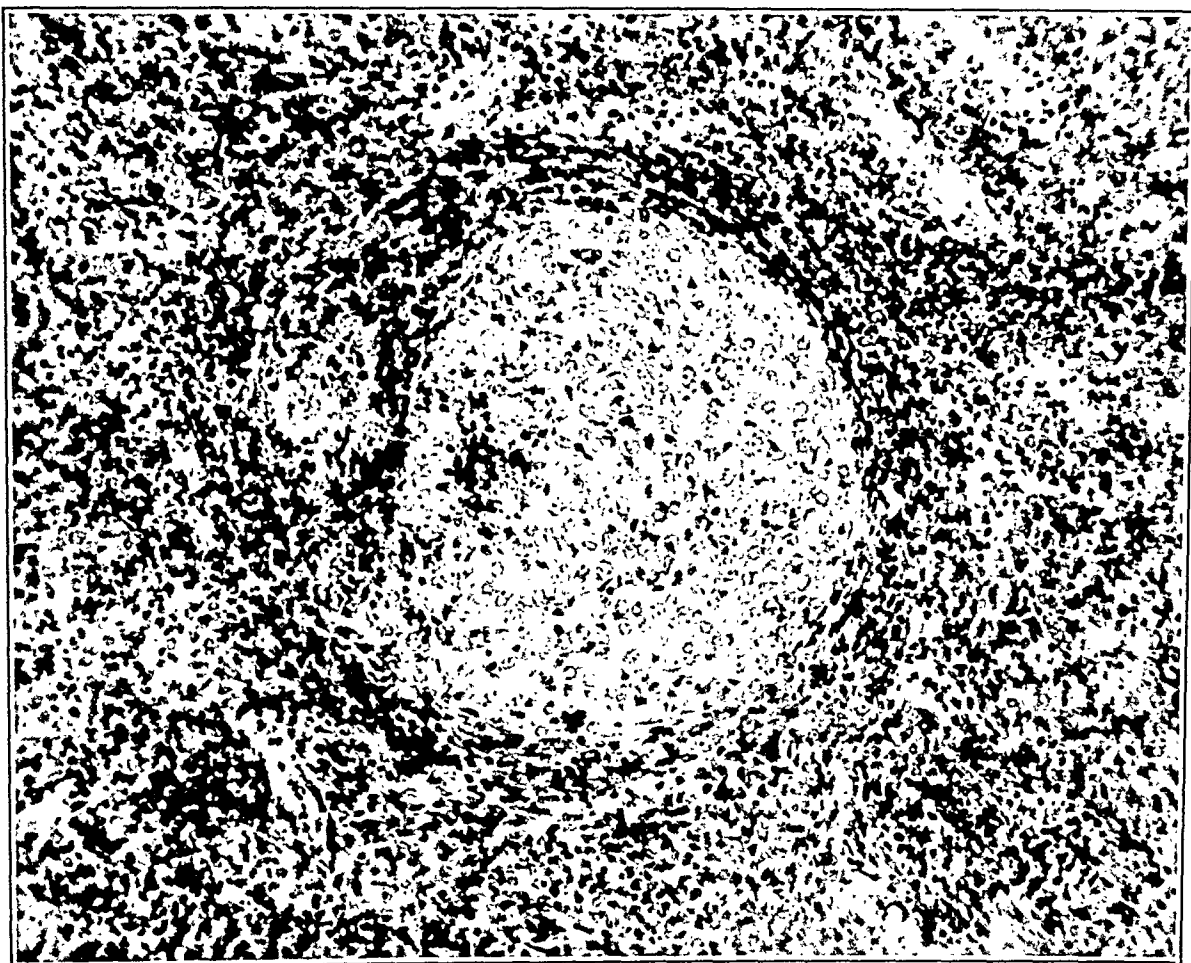
FIG. 4. Focal lesion consisting largely of epithelioid cells occupying a splenic nodule (Guinea pig 18). Animal died forty-four days after intraperitoneal injection of B.C.G. The culture was the fifth generation on glycerinated egg medium. Cultures of acid-fast bacilli were obtained from an emulsion prepared from a portion of this spleen. $\times 120$.



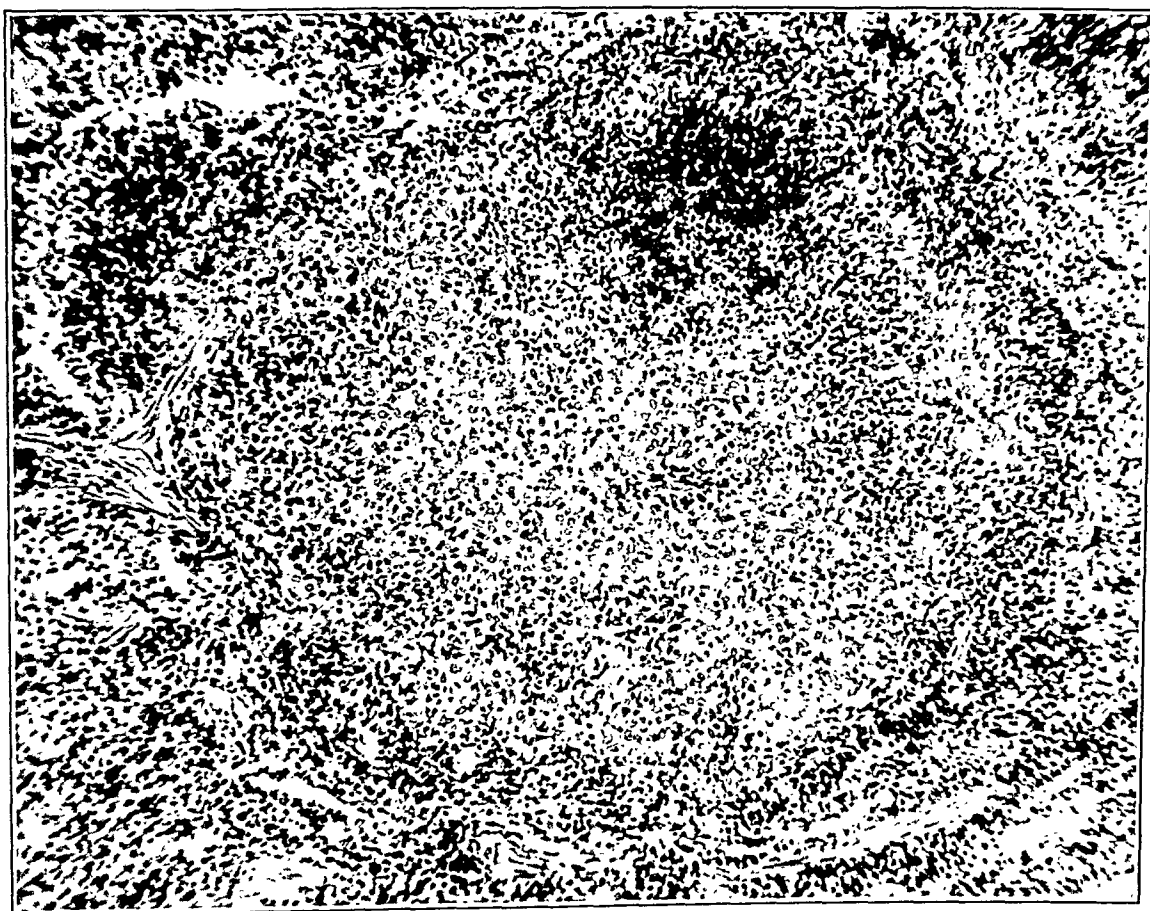
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PLATE 12

FIG. 5. Arterial lesion in lung of normal rabbit after forty-eight hours. The perivascular lymphatics are distended and contain a few clumps of cells. An edematous swelling has produced vacuoles in the media. The entire lower wall is infiltrated with polymorphonuclear leucocytes, and the intima in this region is lifted away from the media by a collection of cells. $\times 90$.

The details of this can be seen in the insert which is a higher power of the lower wall. $\times 250$.

FIG. 6. Necrosis of exudate and alveolar walls, with escape of red blood cells is seen in the lower half of the picture. The alveolar walls in the upper portion are still intact, though many of the nuclei are pyknotic. Normal animal forty-eight hours after introduction of virus. $\times 150$.

TWO SIMPLE METHODS FOR THE SILVER IMPREGNATION OF NERVE FIBERS IN PARAFFIN SECTIONS OF THE CENTRAL AND PERIPHERAL NERVOUS SYSTEM *

NATHAN CHANDLER FOOT, M.D.

(From the Department of Pathology, College of Medicine, University of Cincinnati and Cincinnati General Hospital, Cincinnati, Ohio)

Most pathologists will agree that the methods hitherto devised for the silver impregnation of nerve fibers have been of such a nature as to inspire a certain degree of hesitancy, not to say awe, in the average laboratory worker. Up to the present time it appears that there is but one method available for the silver impregnation of nerves in paraffin sections, that of Rogers,¹ which is of recent date; otherwise one has been thrown back on block or frozen section impregnations which require a certain amount of skill and practice for their execution, and have the tendency to be somewhat capricious and uneven and, hence, to intimidate the beginner in silver technique.

In this paper two methods are described that afford the opportunity for impregnating nerve fibers and fibrils in the brain, cord and peripheral nerve trunks under ordinary circumstances of paraffin sectioning; one of them is a modification of the Ramon y Cajal block impregnation and the other is a practically unmodified Rogers technique. Rogers designed this for the demonstration of non-medullated nerve terminals, especially motor endings. All that has been done here is to apply his procedure to the central nervous system and to try a few experiments with fixatives that he had not mentioned.

THE SILVER NITRATE METHOD

Fixation: After experimenting with about eight different types of fixative, it has been found that the best results are obtainable with a Carnoy's solution that contains no mercury and is made up of absolute alcohol 6 parts, chloroform 3 parts and glacial acetic acid 1 part. The chloroform and alcohol may be kept mixed and the acid added in the proper proportion when it is desired to fix tissue, other-

* Received for publication April 11, 1932.

wise the mixture becomes rapidly converted into ethyl acetate if the acid be added immediately and the fluid kept in stock. This fixative dissolves out the lipids from the nervous tissue and leaves the nerve fibers slightly shrunken but of uniform caliber and with an advantageous index of refraction, so that they stand out clearly. The tissue is fixed for 24 hours, transferred to absolute alcohol for an hour or so and then run through chloroform and chloroform-paraffin into paraffin.

Pretreatment: The sections are deparaffinized with xylol and absolute alcohol in the usual way and they are then left for 1 to 12 hours in a mixture of pyridin 2 parts to glycerol 1 part. They are then washed in 95 per cent alcohol, followed by distilled water, to remove most of the pyridin. A trace of this does not appear to make any difference in the ultimate result.

Impregnation: The slides are then immersed in 10 per cent aqueous silver nitrate and the staining box set on the warm-plate of an incubator run at 37°C for 12 hours or so, the box being covered to prevent evaporation. They are then washed in 2 changes of distilled water. The impregnating fluid may be used repeatedly and kept in the incubator.

Development: The sections are developed for 20 minutes in a 5 per cent neutral formalin solution containing 0.5 per cent pyrogallol, in which they turn yellowish brown. They are then washed at the tap. The developer is prepared fresh for each box of slides.

Toning: Two toning solutions are available: for general purposes a 1:500 aqueous gold chloride is used; added nuclear precision with less intense glial impregnation is obtained if 2 per cent glacial acetic acid be added to this. Five minutes' toning is sufficient to replace silver with gold. The solution keeps for some time and may be used until its effects are visibly weakening.

Intensifying: This important step, devised by Laidlaw,² consists in further reducing the gold by means of immersion for 5 minutes in oxalic acid; in this case it has been found best to add formalin, so that a 1 per cent neutral formalin solution contains 2 per cent oxalic acid. The sections, which have become blackish, grayish or brownish in the toning bath, turn a pleasant violet in this intensifier. They are then well washed at the tap, to prevent precipitating out sulphur from the hypo bath that follows. The intensifying bath may be used repeatedly over long periods of time.

Fixing and Mounting: The sections are fixed for 5 minutes in a 5 per cent aqueous solution of sodium thiosulphate, washed at the tap, dehydrated in ascending percentages of alcohol, run through xylol and mounted in Canada balsam.

THE ROGERS TECHNIQUE

Fixation: Neutral formalin has been found to be the best, which is a great advantage to the routine pathologist; the fixative employed in the preceding method may also be used. Rogers recommends dehydrating the tissue, after washing, in ascending percentages of alcohol to which 1 to 2 per cent of strong ammonia has been added, then placing it in pure absolute alcohol for a few hours and embedding as usual. While this gives excellent results in mixed tissues, it is found to cause some swelling and distortion in brain sections and it is not necessary in any event for the demonstration of nerve fibers unless their end-plates are to be brought out. The modified Carnoy fixative gives good results where coarser, medullated fibers are to be shown; neutral formalin being better for finer, non-medullated fibers.

Pretreatment: After deparaffinizing, the sections are placed in 95 per cent alcohol with 2 per cent strong ammonia for 12 hours or longer. This step is very important as it alkalinizes the sections, which are then rinsed briefly in 80 per cent alcohol and transferred directly to body-warm 40 per cent aqueous silver nitrate for 20 minutes in the incubator. This fluid may be used repeatedly over long periods if it is kept covered to prevent evaporation and contamination.

Combined Impregnation and Development: The sections are next briefly rinsed in distilled water and flooded with 20 per cent neutral formalin for 5 minutes, coming from this to 5 per cent neutral formalin. The staining box is then set on a table, another one is filled with *fresh* 20 per cent neutral formalin and placed nearby and a third is inverted and set between the two. The sections are removed one by one from the weak formalin, blotted briefly on filter paper to remove excess formalin and laid on the inverted box, where they are flooded with a few drops of diammoniacal silver from a dropping-bottle. This solution is prepared by adding strong ammonia drop by drop to 20 cc. of 20 per cent silver nitrate solution until the resulting precipitate is just dissolved. Then an excess of 10 drops of ammonia

is added and the water-clear mixture diluted with 20 cc. of distilled water and poured into a 50 cc. dropping-bottle where it may be kept until it is used up.

Three slides fill the space on the inverted staining box and after each has been flooded in turn with 4 to 5 drops of the silver solution, the first one is blotted and placed directly in the 20 per cent formalin, while another section is removed from the weak formalin, blotted, flooded with diammoniacal silver and left in the place of the first until the turn comes round. The second section is then removed, blotted, flooded and set in the strong formalin with the first and so on until all the sections have been treated in rotation and are in the developer where they turn rusty orange, and where they should remain another 5 minutes to ensure complete development. It is found that the time required to flood, blot and transfer three sections in this manner is just sufficient for proper impregnation — about 1 minute in practice.

Subsequent Treatment: Toning is accomplished by 15 minutes' treatment in a 1:300 gold chloride bath, acidified with 2 per cent glacial acetic acid. Intensification, fixing, dehydration and mounting are precisely similar to that which has been described in the case of the preceding method.

Precautions: The reader is cautioned that the diammoniacal silver solution is made up *without the addition of sodium hydroxid or sodium carbonate* usually practised in Bielschowsky or other methods. In carrying out the Rogers method one is dealing with very concentrated silver solutions and should, therefore, protect the hands with rubber gloves and the clothing and shoes as well from the action of these. Rubber gloves, old shoes and a capacious laboratory apron or coat can not be too highly recommended.

BRIEF RESUMÉ OF STEPS

Silver Nitrate Method

1. Pretreatment with pyridin-glycerol for 1 to 12 hours.
2. Wash in 95 per cent alcohol followed by distilled water.
3. Impregnate 12 hours or longer in 10 per cent silver nitrate in incubator.
4. Wash in 2 changes of distilled water.
5. Develop in pyrogallol-formol 20 minutes.

6. Wash at tap and tone 5 minutes in gold bath.
7. Intensify in oxalic acid-formalin 5 minutes after washing at tap.
8. Wash well at tap and fix in hypo for 5 minutes.
9. Wash, dehydrate, clear and mount in Canada balsam.

Rogers' Method

1. Pretreatment with ammoniated alcohol 12 hours or more.
2. Rinse in 80 per cent alcohol and impregnate in 40 per cent silver nitrate in the incubator for 20 minutes. Rinse in distilled water.
3. Treat with 20 per cent formalin 5 minutes, transfer to 5 per cent formalin.
4. Reimpregnate sections one by one, after blotting off formalin, with diammoniacal silver; blot and develop. Do not wash!
5. Develop in 20 per cent formalin 5 minutes, or until all sections are a rusty orange.
6. Wash in distilled water and tone in gold bath for 15 minutes.
7. Wash at tap and intensify, fix and mount as in preceding schedule.

REMARKS

With these two methods at one's disposal a great deal can be done in connection with the study of nerve fibers of different types. For the brain and cord (Fig. 1), as well as for large peripheral nerve trunks like the sciatic or femoral (Fig. 2), the silver nitrate procedure is best adapted; for the demonstration of the finest neurofibrillae, such as the non-medullated fibers in the nuclei of the medulla or those in the superficial cortex (Figs. 3 and 4) the Rogers method is the better.

The silver nitrate technique is about as simple as a silver impregnation could be; it gives a clear-cut impregnation of the nerve fibers which take on a light red or fox-red color and stand out from the very fine, violaceous glia fibrils. The nerve cells, ganglion cells and the like, tend to take on the same reddish tone as do the neurones; the neuroglia cells, particularly the oligodendroglia cells, impregnate grayish to blackish. This fact may conceivably be of use in certain investigations. The method is not suitable, nor is it intended for the demonstration of neuroglia cells and their processes.

On the other hand, it impregnates nerve fibers very successfully and few of them elude it; the basket fibers around Purkinje cells and the fibers in the granular zone of the cerebellum are best brought out in sections toned with acid gold. The nuclear detail in the cells is very good; if they tend to overimpregnate, the acid gold bath will correct this at the cost of slight loss of general intensity of the fiber impregnation. Although this method impregnates the finest fibrils in sections of nervous tissue, they are apt to be obscured somewhat by the presence of neuroglia fibers. In this case, one resorts to Rogers' method which is more selective for the finer elements.

In that method these are brought out black on a less densely impregnated background, the details of the cellular elements and of their nuclei may not be as sharply picked out as in the preceding method, but they may be very adequately shown. Following fixation in the modified Carnoy solution Rogers' method gives good impregnation of the coarser fibers, the color scheme tending to be more on the red, less on the black side. The reader may experiment with the two methods and ascertain which of them suits his particular needs.

It will be noted that, after repeatedly stressing the importance of using equimolar solutions I have not employed them here; the reason is quite simple — they do not work as well as do the concentrations herein noted, which are the result of considerable experimentation. Naturally, this does not apply to the Rogers method which has been taken over practically unchanged.

SUMMARY

Two methods are described for impregnating nerve fibers in paraffin sections; one is a modification of the Ramon y Cajal silver nitrate block impregnation, while the other is a practically unmodified Rogers technique. The former is best suited to the demonstration of fibers in the central nervous system and in large peripheral nerve trunks in their relation to supporting structures: the latter is an application of Rogers' method for demonstrating terminal nerve endings for the more general purpose of bringing out non-medullated fibrils in the central nervous system. Both methods are designed for general use in the histological laboratory on tissue that has been fixed not over five hours postmortem. The silver

nitrate method is well standardized and should produce reasonably uniform results; the Rogers method may entail a modicum of practice in the step whereby the sections are reimpregnated one by one in the diammoniacal silver, otherwise it presents no pitfalls for the unwary.

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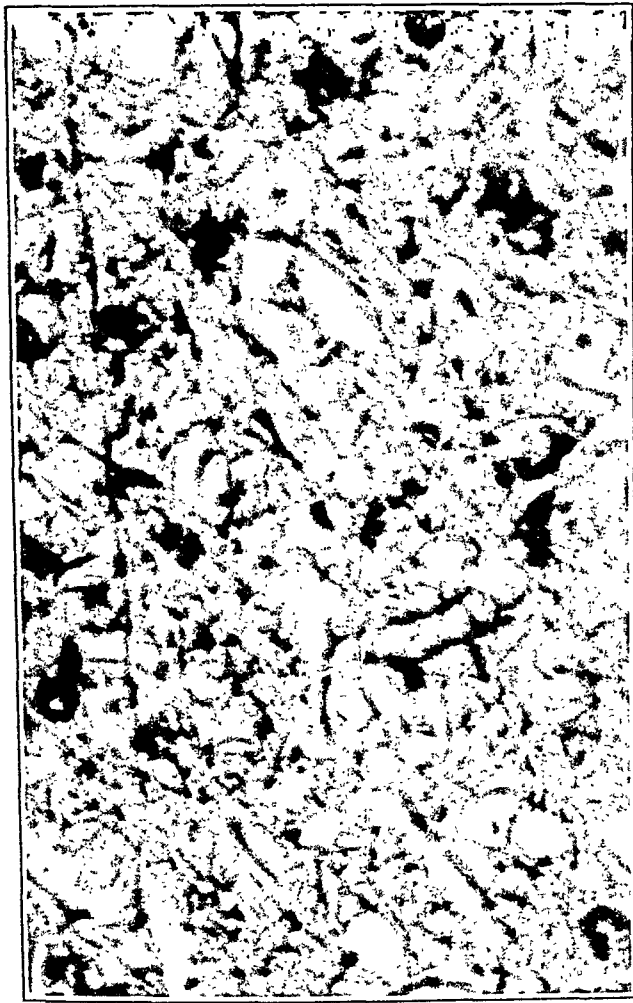
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2. Laidlaw, G. F. Silver staining of the skin and of its tumors. *Am. J. Path.*, 1929, 5, 239.

DESCRIPTION OF PLATE

PLATE 121

All photomicrographs were taken at 800 diameters by Prof. J. B. Homan of our Department of Medical Art, with the assistance of the author.

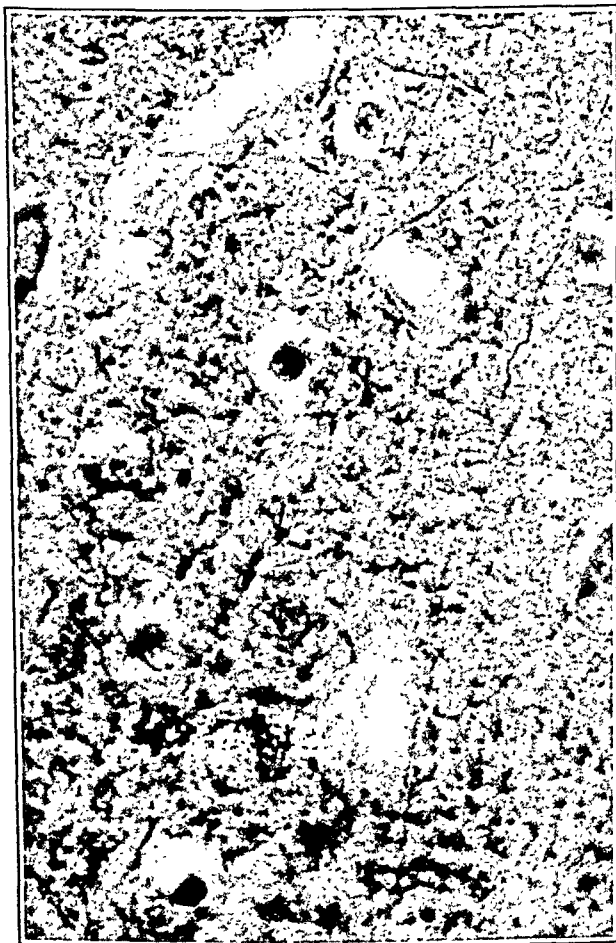
- FIG. 1. Silver nitrate method, fibers in cortical white matter.
- FIG. 2. Silver nitrate method, fibers in the femoral nerve. Most of the coagulation phenomena seen in sections fixed by other methods that do not extract the myelin are done away with here. The "funnels" remain.
- FIG. 3. Rogers' method. Fine fibrils in gray matter of cortex.
- FIG. 4. Ganglion cells and fine fibrils from the region of the floor of the fourth ventricle. Rogers' technique.



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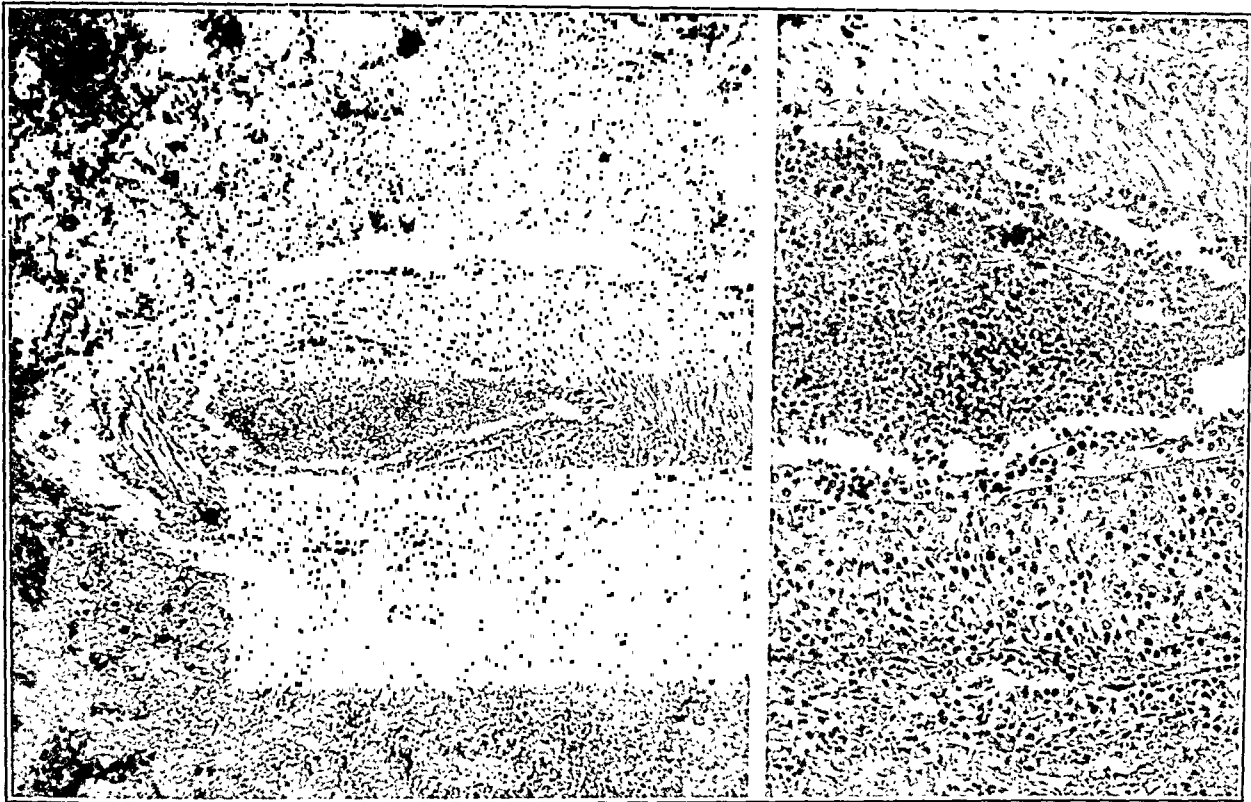
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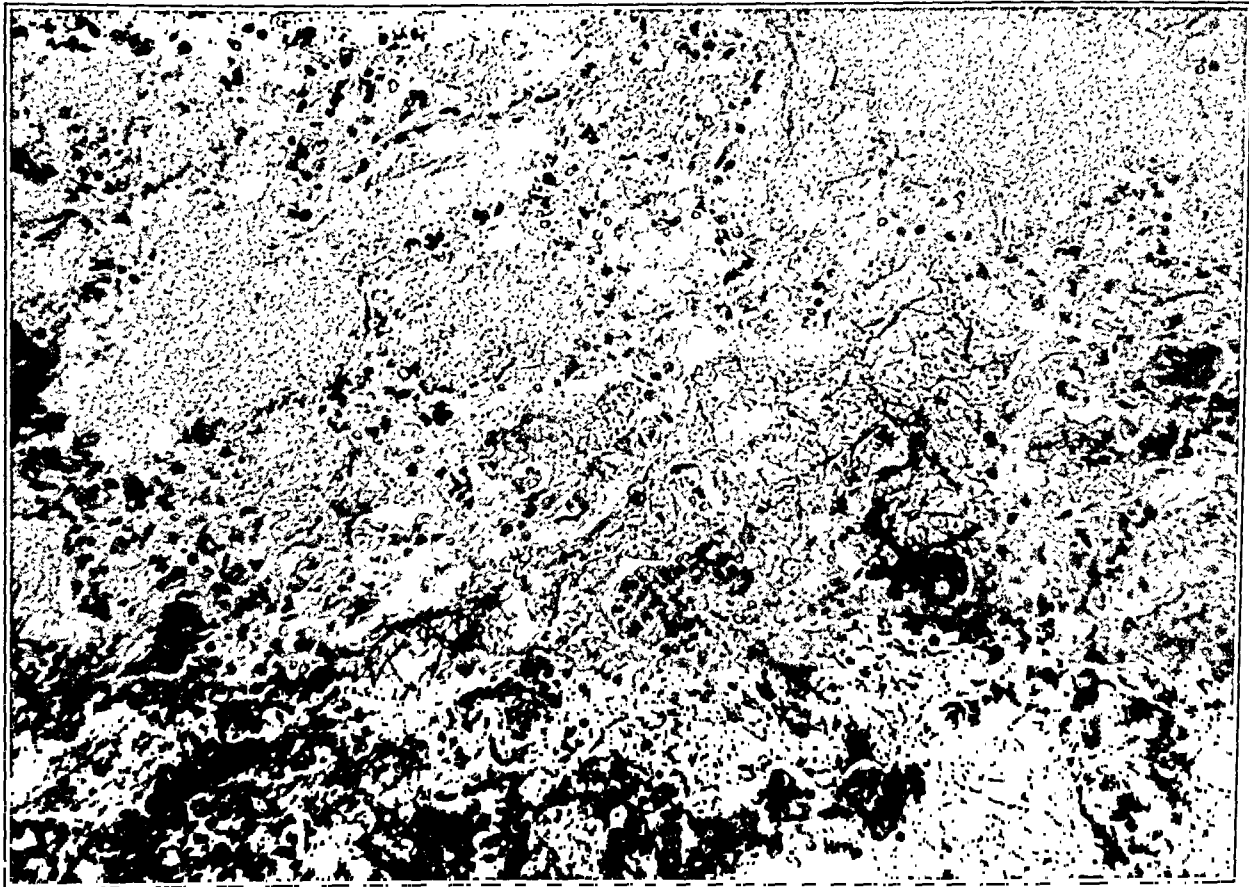


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Silver Impregnation of Nerve Fibers



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mented, which is of interest because it is not generally known that silver impregnation will succeed after fixation in other than one or two of the commoner formalin or bichromate methods. It was to determine the possible range of fixatives at one's disposal that the work reported in this paper was undertaken prior to reading Davenport's articles.

TECHNIQUE

The essential for this experiment was to have a standard material and a standard impregnation that would be as simple and constant as possible. Accordingly, a few millimeters of femoral nerve was removed not later than two hours postmortem from human subjects, while they were still warm. As the impregnation, an adaptation of the familiar Ramon y Cajal block impregnation was used, in which the tissue was first sectioned in paraffin, mounted on slides and deparaffinized in the usual manner, after which the sections were treated with pyridin 2 parts, and glycerol 1 part, for 1 to 12 hours. They were then washed in 95 per cent alcohol, followed by tap water and 2 changes of distilled water, and impregnated for 12 to 24 hours in a 10 per cent aqueous silver nitrate solution in the incubator at 37° C.

After a wash in distilled water they were developed for 20 minutes in a mixture of pyrogallol and formalin, 5 parts each to 100 parts distilled water. It was later found that the pyrogallol could be reduced to one-tenth this concentration without materially changing the picture. After developing, the sections were washed at the tap and toned for 5 minutes in a 1:500 aqueous solution of Merck's "acid red" gold chlorid. They were again washed at the tap and intensified for 5 minutes in a mixture containing oxalic acid 2 per cent and formalin 1 per cent in distilled water, after which they were well washed at the tap and fixed in 5 per cent sodium thiosulphate for 5 minutes, washed and then dehydrated, cleared and mounted in Canada balsam in the usual way.

FIXATIVES AND RESULTS

The fixatives used may be grouped into four general classes:

(1) *Alcoholic Fixatives*: (a) 95 per cent alcohol; (b) absolute alcohol; (c) ammoniated absolute alcohol, containing 2 per cent

THE EFFECT OF DIFFERENT TYPES OF FIXATION ON THE SILVER IMPREGNATION OF PARAFFIN SECTIONS OF PERIPHERAL NERVE *

NATHAN CHANDLER FOOT, M.D.

*(From the Department of Pathology, University of Cincinnati College of Medicine, and
Cincinnati General Hospital, Cincinnati, Ohio)*

While experimenting with a method for impregnating paraffin sections of nervous tissue with silver, as already reported elsewhere (Foot¹), it was found that the results obtained in the case of peripheral nerve trunks varied widely with the fixative used. So wide was this variation and so striking, that it is thought to be of sufficient interest to be reported. Some work has been done along this line by Davenport,² who experimented upon blocks of cat's spinal cord, using a variety of fixatives and a standard silver nitrate impregnation (Ramon y Cajal), and bending his efforts toward determining the most favorable ammonia concentration in the alcoholic fixing fluid, together with the effect of extraction of lipins from the tissue by means of adjuvants to the alcohol, such as chloroform and pyridin. He found that the optimum impregnation was obtained after the use of alkaline fixatives that, at the same time, extracted the lipins and thus permitted complete penetration of the silver salt. Furthermore, he investigated the effect of varying the proportion of pyrogallol and formalin in the developer and found the former to be the essential ingredient, as formalin did not reduce silver nitrate although it does reduce the double ammonia salts of silver. His second paper³ dealt with acid fixatives, such as sulphosalicylic acid, trichloroacetic acid, Hofker's fluid and Carnoy's fixative. There was less shrinkage of the tissue than was the case when ammoniated alcohol had been used, but during the necessary alkalization, washing and silvering, the acid-fixed tissue ultimately shrank more than the controls, Carnoy's solution bringing about the maximum shrinkage noted. This he determined by measuring the blocks before and after fixing and impregnating. Successful silver impregnation was obtained after the use of all the fixatives with which he experi-

* Received for publication May 26, 1932.

as do the neurones, which is also true of the fine fibrils that are embedded in the connective tissue of the epineurium. The nuclei of the tissue are all well shown. There is not much fundamental difference between the fixatives in this group, excepting that the modified Carnoy's solution is the most successful while the 95 per cent alcohol gives poor results. The former, containing acetic acid, tends to offset the shrinkage of the fibers that results in straight alcohol fixation. There is, therefore, a graded scale of excellence between the modified Carnoy's solution at the top and stock, unalkalinized alcohol at the bottom.

Formalin Fixation: Neutral formalin is commonly supposed to be the ideal fixative for silver impregnations, but the results obtained in this experiment with formalin alone, or in combination with ammonium bromid or other ingredients, are not in accord with this conception in so far as peripheral nerves are concerned. One sees at once that the axones show very marked irregularity of caliber and outline (Fig. 2), although they are metachromatically impregnated and stand out prominently by reason of their fox-red color. Here and there one notes fusiform, or beaded swellings of the axones, in which the substance of the fibers is pale; elsewhere the neurones may be markedly contracted and delicate. Thus the fibers show an alternation between what may be considered quasi normal caliber and either fusiform swelling, or marked shrinkage. The perineural fibers are well brought out, as is the connective tissue of the nerve trunk. The nuclei are beautifully impregnated, the funnels stand out well and there is a wealth of detail in the myelin sheath, which stains yellowish to orange. One finds longitudinal fibers and a very intricate, wide-meshed web of tiny fibers that radiate through the sheath and make a very pleasing, though probably deceptive picture. In the case of the formalin-bromid fixative the connective tissue becomes too prominent and darkly impregnated, the neurones are even more irregular in caliber and there is an orange network in the myelin sheath, as in the preceding case. The reticulum of the fibrous sheath and of its vessels is well demonstrated, in sharp contrast to the alcohol-fixed specimens.

Chromic Fixation: In this case one would think that one was dealing with quite another histological structure, so strikingly different is the picture obtained (Fig. 3). The myelin sheath has been impregnated a reddish brown, it shows innumerable radiating, frond-like

strong ammonium hydroxid; (d) alcohol 6 parts, chloroform 3 parts and glacial acetic acid 1 part (omitting the mercury bichloride that is usually an ingredient of Carnoy's solution).

(2) *Formalin Fixatives*: (a) Neutral 10 per cent formalin, and (b) Ramon y Cajal's "bromformol" with 15 per cent formalin and 3.5 per cent ammonium bromid.

(3) *Chronic Fixatives*: (a) Zenker's fluid, with 5 per cent mercuric chlorid, 2.5 per cent potassium bichromate and no sodium sulphate, adding 5 cc. of glacial acetic acid to every 95 cc. of the mixture at the time the blocks are fixed; (b) Helley's fluid, which is identical with Zenker's except for the substitution of 10 cc. of strong formalin in the place of the 5 cc. of acetic acid and the reduction of the amount of stock solution from 95 cc. to 90 cc.

(4) *Acid Fixatives*: This group was represented by Bouin's solution (water-saturated picric acid 15 parts, strong formalin 5 parts and glacial acetic acid 1 part).

Alcoholic Fixation: Nerve trunk fixed in the alcoholic solutions shows the axones to be somewhat shrunken, but of a nearly uniform caliber. They are reddish to yellowish brown and stand out with remarkable distinctness. The rather complicated pictures to be described later in connection with the myelin sheaths are not noted; there is an outer envelope with a space that contains a few bits of granular, or globular debris and the shrunken funnels of Schmidt, which are usually collapsed about the neurone as dark swellings. In the modified Carnoy's fixative, however, they are more clearly brought out and less shrivelled. The nodes of Ranvier are well demonstrated. There is a marked simplicity to the histological picture, which is to be attributed to the extraction of coagulable or argen-taffin lipins from the myelin sheath. One may find areas where extraction has been less complete and where bubble-like outlines and granular masses may be found to be impregnated. In those portions of the section where the extraction has run its course, this layer of the sheath is almost clear and empty, save for the Schwann cells. The connective tissue elements are not particularly well impregnated and tend to be slaty or violaceous in color, which is an advantage in that the neurones stand out in contrast by reason of their brownish tint (Fig. 1).

The leashes of delicate fibrils that parallel the neurones in the nerve trunk and lie between them take on the same brownish hue

alcohol is its tendency to shrink the axones, but this may be offset by the addition of acetic acid. The use of such a fixative demonstrates the defects of fixatives that may combine with, or coagulate the lipins — for in such a case histological details are brought out that probably do not exist in fresh tissue.

It is surprising to find formalin combinations so disturbing to the neural histology in view of their extensive use in fixing nervous tissue and in connection with silver techniques; it is disappointing to discover how much they distort the actual nerve fibers while they give such excellent results in the case of their adnexae. The more concentrated formalin in Ramon y Cajal's fixative seems to increase this distortion and this is not corrected by adding picric and acetic acid, and decreasing the percentage of formalin, as in Bouin's solution. Further proof of this action on the part of formalin may be obtained through the observation of nerve fibers impregnated by Rogers' method,⁴ in which the impregnation is very fine but the outline of the neurones actually scalloped, after fixation in formalin or Bouin's fluid. One immediately wonders how accurate are the pictures produced in non-medullated nerves and neural end-organs under such circumstances.

The optimum fixative, then, would be one which would give: (a) as little distortion of the nerve fibers as possible; (b) metachromatic results, so that nerves and connective tissue fibers have different colors; (c) good nuclear impregnation and (d) good impregnation of the connective tissue elements without unduly emphasizing them. Thus far, in connection with this simple silver nitrate impregnation, the alcohol-chloroform-acetic acid mixture seems most nearly to accomplish these ends.

SUPPLEMENTARY WORK

As it would be interesting to determine what result, if any, would be obtained through the use of Mallory's preliminary "bleach" of permanganate of potash and oxalic acid, sections fixed in the four types of fixative were subjected to treatment with weak alcoholic iodine, weak sodium thiosulphate to remove this and then treated with 0.25 per cent aqueous potassium permanganate, followed by 5 per cent oxalic acid. They remained in each of these solutions for 3 minutes with washes in water between each step. It was found that the bleach almost invariably tends to loosen the sections from

fibrils that appear to spring from the pinkish neurone and run out to the sheath of Schwann. The funnels are embedded in this network and are almost indistinguishable, the neurones are of fairly even caliber, pinkish color and very well and evenly brought out, while the connective tissue of the sheaths is violaceous to grayish. In cross-sections of nerve fibers the familiar "Sonnenbilder," or rosettes, are prominent. The picture is very pleasing and appears to be very accurate at first glance, but one may well believe that it is, on the contrary, quite deceptive.

In the chromium-mercury methods one is forced to use iodine and sodium thiosulphate to remove the mercuric chlorid crystals, before proceeding with the impregnation. Experiments with sections fixed in other types of solution failed to produce similar pictures if iodine and hypo were used, so that we may suppose that the effect noted in the myelin sheath is due to the action of chromium or mercury and not to the iodine treatment. Chromicizing formalin- or alcohol-fixed sections for 24 hours in the incubator with a mixture of equal parts of 10 per cent chromic acid and saturated aqueous bichromate of potash fails to produce similar pictures, however, so that these salts must, apparently, exert their effect only on fresh, unfixed tissue. It should be noted, however, that formalin fixation produces somewhat similar pictures; it seems as though the coagulative action in this case does not proceed as far, or act so strongly.

Acid Fixation: Material fixed in Bouin's solution gives results that accentuate connective tissue elements at the expense of the nervous. The neurones are irregularly, often very lightly impregnated (Fig. 4) and there is more swelling and distortion of these than was the case after formalin fixation, long stretches of nerve fiber being swollen to the capacity of the sheath and of greater diameter than in any other case. The illustration shows this only in part. They are often so faintly impregnated that they are difficult of identification. The nuclear and connective tissue detail is very good.

REVIEW AND DISCUSSION OF THE FINDINGS

Reviewing these results we find that the most reliable fixation for the purpose of silver nitrate impregnation corresponds with Davenport's experience — it should be one that extracts the lipins and permits the penetration of the silver solution. The disadvantage of

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DESCRIPTION OF PLATE

PLATE 122

All four photomicrographs were made from sections of human femoral nerve, removed while the body was still warm. The impregnation is identical in each case, the only difference being in the fixative employed. The magnification represents about 400 diameters. The photomicrographs were taken by Prof. J. B. Homan of our Department of Medical Art, with the author assisting.

FIG. 1. Silver impregnation following fixation in alcohol-chloroform-acetic acid. The heavy black cables are the nerve fibers, or neurones, which are somewhat shrunken but of fairly regular caliber. There is a paucity of detail in the myelin sheath, the funnels are partially shown and the Schwann cells, with their processes, are easily recognized.

FIG. 2. Silver impregnation following fixation in neutral formalin. The neurones show irregular bulging and are sometimes much shrunken; there is a very irregular fixation. The details of the myelin sheath are somewhat increased, as compared with the preceding picture. Note the beaded nerve fiber at the left of the field.

FIG. 3. Silver impregnation following fixation in Zenker's fluid. The nerve fibers are more uniformly fixed and more contracted. There is a wealth of detail in the myelin sheath, caused either by coagulation of the lipins or by some chemical combination between the salts of the fixative and the silver salts.

FIG. 4. Silver impregnation following fixation in Bouin's fluid. Here the nerve fibers are difficult to follow in their course; they show areas of apparent dissolution, one of which is at the right of the field. The details of the myelin sheath are not well brought out, while those of the longitudinal fibers of the endoneurium are excellently demonstrated.

the slides, unless they have been very carefully mounted and dried. This has been a troublesome feature often noted in reticulum impregnation and it is probable that it may be overcome by the use of Masson's formalin-hardened gelatin method of mounting sections.⁵

The net result after using the bleach is not very striking; the sections show some increase in precision of detail at the expense of depth of impregnation. The Zenker-fixed sections show much more delicate pictures, except that they have become monochrome (brownish) without the desirable metachromasia. The Bouin-fixed sections are even worse than before. It would seem that there is nothing to gain and much to lose through the use of the bleach in this particular connection. It is to be noted that this does not apply to connective tissue impregnations, merely to those of nerve trunks.

SUMMARY

1. Experiments on four representative groups of fixatives, used on standard material (normal human femoral nerve) and followed by a standardized and very simple method of silver impregnation, show that the results differ almost directly as the number of fixatives used. Although the variation within a given group of similar fixatives is slight, those between any two groups are decidedly marked.

2. Alcohol fixation tends to remove lipins from the myelin sheath and thus affords the clearest impregnation, possibly the most veracious. The best alcoholic solution is a combination of alcohol, chloroform and acetic acid.

3. Formalin fixation, even when neutral formalin is used, causes marked distortion of the neurones and brings out a certain amount of what may be considered to be extraneous detail, caused by the coagulation or chemical alteration of the myelin.

4. Chromate fixation, while it affords very precise and clear pictures, demonstrates even more histological detail, which is probably artefact, owing to a similar, but more pronounced action on the lipins.

5. Fixation in such an acid solution as Bouin's fluid causes an accentuation of the connective tissue elements at the expense of the nervous and gives to the neurones an unduly swollen and transparent appearance without effecting much metachromatic contrast.



1



2



3

Foot



4

Effect of Fixation on Silver Impregnation

PLATE 13

FIG. 7. Guarnieri bodies in the epithelial cells of a small bronchus. Most of the inclusions have a clear halo and indent the nucleus. Normal rabbit forty-eight hours after inoculation. $\times 1000$.

FIG. 8. Unusually dense collection of large mononuclear cells in the lung of an immune animal forty-eight hours after inoculation, completely disguising the pulmonary architecture. $\times 400$.

SILVER IMPREGNATION OF GLIA AND NERVE FIBERS IN PARAFFIN SECTIONS AFTER FORMALIN FIXATION *

HELENOR CAMPBELL WILDER

(*From the Army Medical Museum, Washington, D. C.*)

Silver impregnations of nerve fibers and glia comprise a formidable number of long and tedious processes. Many of these methods give beautiful results, but, as they require special fixatives or lengthy and tedious pretreatment, they are impracticable for use in the majority of laboratories where formalin is the routine fixative. Foot^{1, 2} has obtained satisfactory glial impregnation in frozen sections after fixation in neutral formalin and, with his work as a starting point, I have endeavored to devise a method that would be applicable to paraffin sections as well.

Using the silver diammino hydroxid recommended by Foot,^{2, 3, 4} as a result of his tests of the work of Kubie and Davidson⁵ and his formol-sodium carbonate reducer, I experimented with paraffin sections after formalin fixation. The experiments included application on the slide, before impregnation, of reagents advocated as sensitizers of glia to silver. Ammonium bromid,⁶ ammonium hydroxid,⁷ hydrobromic acid,⁷ pyridin,³ Carnoy's fluid,⁸ and other reagents that have been of value in neurological methods were tried without success. Del Río-Hortega's⁹ results with formalin-uranium nitrate fixation preparatory to silver impregnation of frozen sections led me to precede silver impregnation of formalin-fixed paraffin sections by treatment with uranium nitrate. A differential reaction to silver was immediately apparent. There was no precipitate deposited on the slide and the sections showed argentation of the fibrillar elements; nerve fibers, fibrillar astrocytes, reticulum and collagen were impregnated. After uranium nitrate, silver staining was so intense that it became necessary to use the silver diammino hydroxid at room temperature rather than at 50° C, and to reduce the time from 30 minutes to 20 seconds. Uranium nitrate in 1 per cent solution proved most satisfactory, but exposure of more than 5 seconds inhibited impregnation. The other nitrates were of no value.

* Received for publication July 1, 1932.

The previous experiments were repeated, introducing the additional step of uranium nitrate, because sharper definition of nerve fibers, more complete staining of glial elements, a clearer background, and an elimination of reticulum and collagen were desirable. Hydrobromic acid accomplished the desired results with the greatest consistency and proved of especial importance as a differentiating factor. Substitution of uranium nitrate for the sodium carbonate of Foot's reducer further accentuated the glial fibers. The gold toning of Foot's revised Variant 3⁴ (1: 500 gold chloride, 10 minutes; formalin 5 cc., oxalic acid 0.5 gm., water 100 cc., 10 minutes; 5 per cent sodium thiosulphate, 10 minutes; wash in tap water after each step) tended to clear the background and, where glial fibers showed granular deposits of silver, to give them a more solid and fibrillar appearance. However, the improvement was not consistent and, as it failed to differentiate glia from nerve fibers, it is not included as an essential feature of the stain.

All experiments were carried out on material that had been fixed in unneutralized formalin from two to twenty-four hours after death or removal.

TECHNIQUE

Fixation and Embedding: Fix tissues in 10 per cent formalin, wash in tap water, dehydrate in alcohol, clear in chloroform, and embed in paraffin.

Bromuration: Pass paraffin sections through xylol and graded alcohols, rinse in distilled water, and place in 34 per cent hydrobromic acid for 30 minutes.

Sensitization: Wash in distilled water 10 to 20 seconds and flood the slide with 1 per cent uranium nitrate (sodium free) for 5 seconds or less.

Impregnation: Wash in distilled water 10 to 20 seconds and place for 20 seconds in silver diammino hydroxid:

To 5 cc. of 10.2 per cent silver nitrate add ammonium hydroxid drop by drop until the precipitate which forms is dissolved. Add 5 cc. of 3.1 per cent sodium hydroxid and just dissolve the resulting precipitate with a few drops of ammonium hydroxid. Make the solution up to 50 cc. with distilled water.

Reduction: Wash in distilled water 2 seconds and agitate each

slide separately in the following reducing solution until it ceases to give off a brown cloud:

Distilled water 50 cc., 40 per cent neutral formalin (neutralized with magnesium carbonate) 0.5 cc., 1 per cent uranium nitrate 1.5 cc.

Counterstaining and Mounting: Wash in distilled water, counterstain with eosin, dehydrate in alcohol, clear in xylol and mount in Canada balsam. Argentation frequently allows hematoxylin to be used as a nuclear stain, but bluing must take place in tap water, as ammonia dissolves the silver.

Distilled water is used in the preparation of all solutions. The uranium nitrate solution and the 10.2 per cent silver nitrate keep indefinitely and the silver diammino hydroxid keeps for a week or more in amber, glass-stoppered bottles. The impregnating and reducing solutions retain their activity in Coplin jars for two days. The hydrobromic acid may be kept in a Coplin jar and used repeatedly for an indefinite time. It is important that the ammonium hydroxid be kept in a well stoppered bottle.

RESULTS

Ganglion cells, nerve fibers, glia cells and their processes are black. Tissues fixed twenty-four hours after death or removal show excellent impregnation of nerve fibers and fibrous astrocytes, but the processes of protoplasmic astrocytes and oligodendroglia cannot be demonstrated when more than six hours have elapsed before fixation. In fresh fixed tissue all the fibers are sharply defined, but tend to become granular when fixation is less prompt. Although differentiation between nerve fibers and glia must be on a morphological basis, the method has the advantage of being quick, simple, and applicable to paraffin sections of formalin-fixed tissue.

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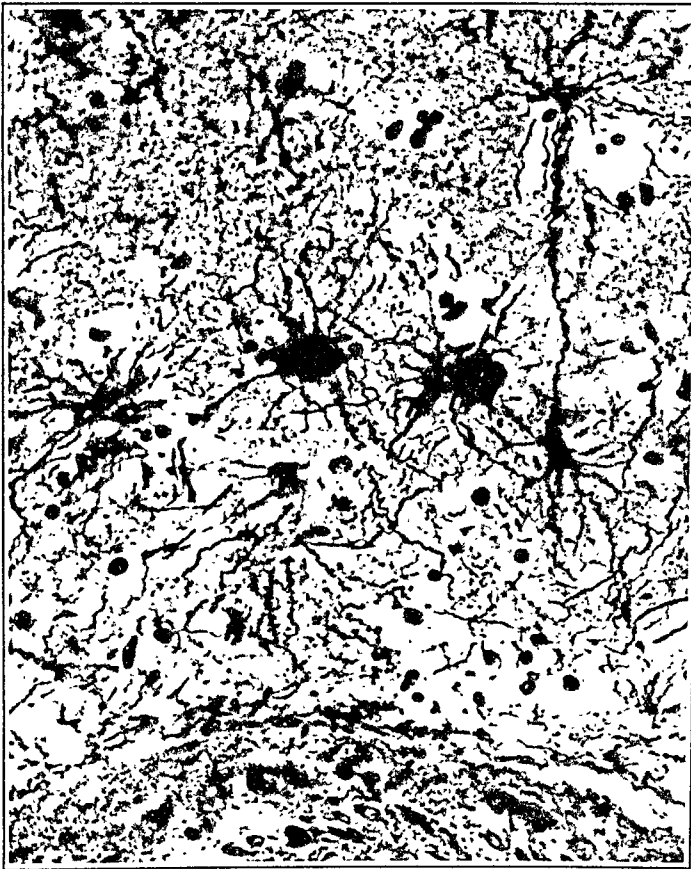
 DESCRIPTION OF PLATE

PLATE 123

- FIG. 1. Fibrous astrocytes with pedicles to a blood vessel in a medulla fixed twenty-four hours after death. $\times 600$.
- FIG. 2. Large astrocytes around a vessel in cerebral arteriosclerosis. The tissue was fixed twenty-four hours after death. $\times 475$.
- FIG. 3. Oligodendroglia in a cerebral cortex fixed six hours after death. $\times 600$.
- FIG. 4. Fibrous astocytoma fixed two hours after death. $\times 600$.
- FIG. 5. Fibrous astrocytes in a spinal cord fixed twenty-four hours after death. Nerve fibers appear in cross-section. $\times 475$.
- FIG. 6. Bipolar cells in a spongioblastoma multiforme fixed ten hours after death. $\times 475$.



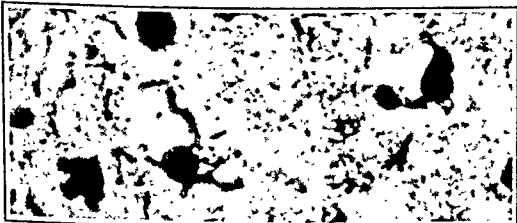
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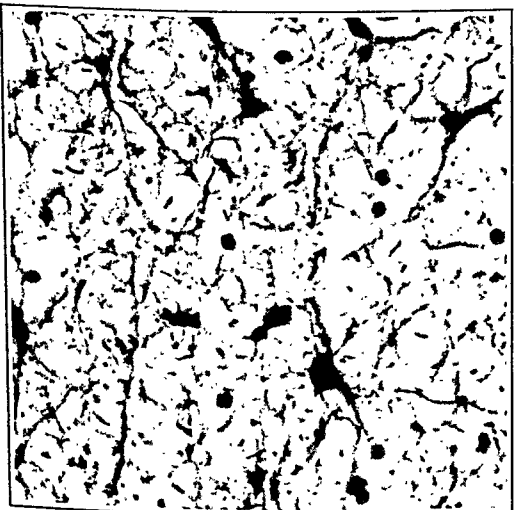
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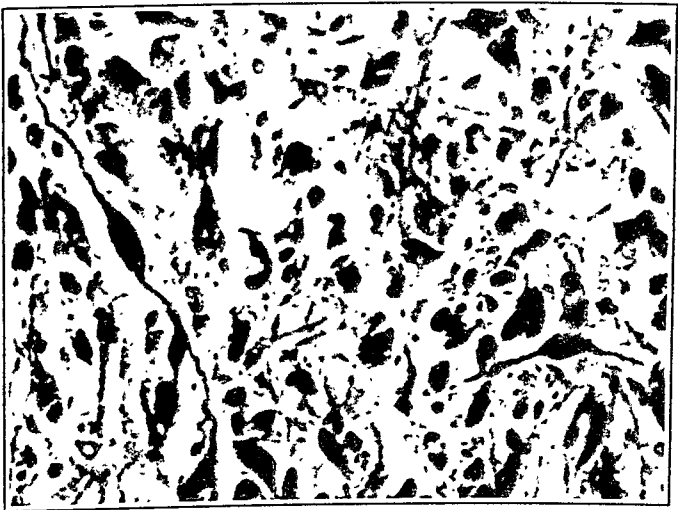
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5



6

Wilder

Silver Impregnation of Glia and Nerve Fibers

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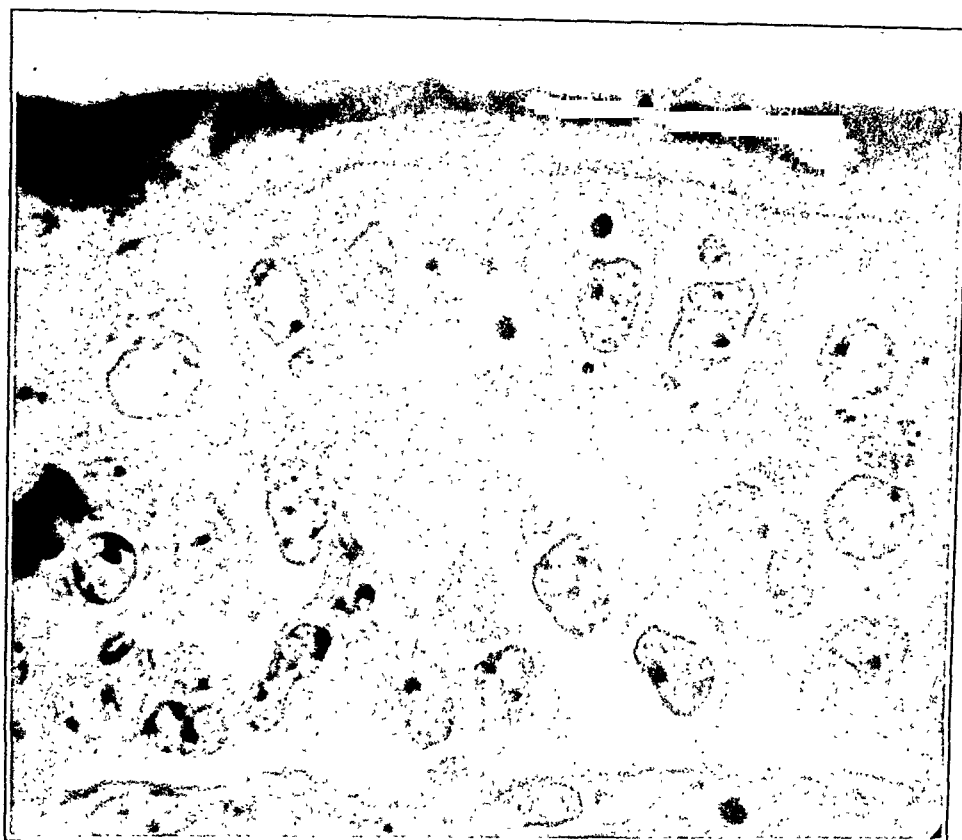
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* Abstract of paper presented at the meeting of the American Association of Pathologists and Bacteriologists held at Philadelphia, Pa., April 28 and 29, 1932.

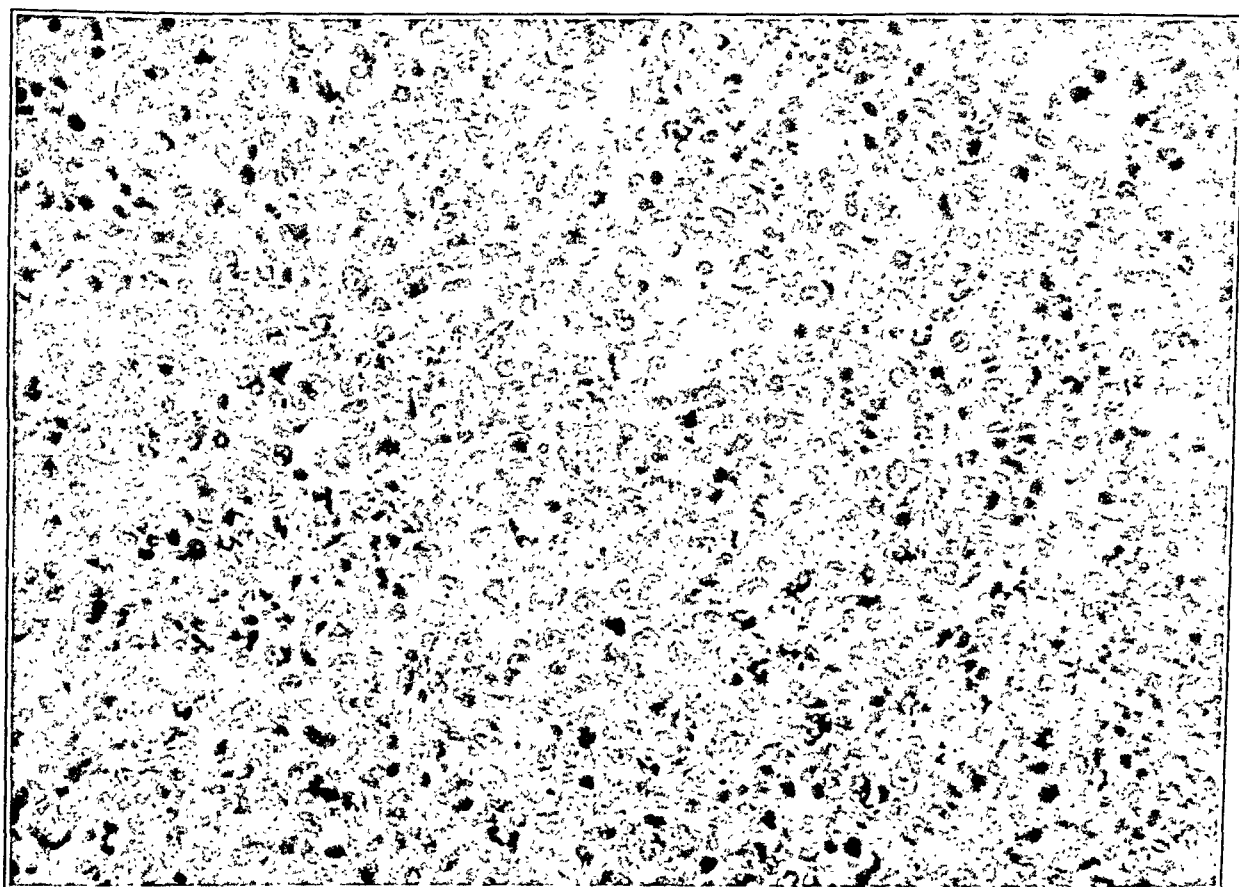
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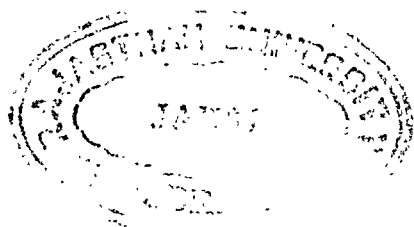
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In 1929 Yater,⁶ in a splendid contribution on variations in the right atrial venous valves, found four examples in 120 hearts which he examined, an incidence of 3.3 per cent. In comparing my findings with those of Yater, I feel certain that I must have overlooked some cases incident to carelessness in removing and opening the heart. In the usual routine methods employed in these procedures little care is exercised in preserving the inferior vena cava, and, when the right auricle is opened, these delicate, thread-like structures are readily severed or torn away. Since employing more careful methods, consisting of looking first into the inferior vena cava, and then so manipulating the scissors or knife when opening from cava to cava as to avoid any possible damage to structures of this character, I have found such a network present and intact, which could easily have been destroyed had not such precautions been applied.

Interesting and peculiar clinical phenomena connected with the reported cases of "Chiari's network" have been observed, although most of them were merely interesting accidents, since in almost all of the reported cases the networks were of no significance clinically. In one of his cases Chiari found that a thrombus had formed on the network, and this was the apparent source of a large pulmonary embolus from which the patient died. Haas,⁷ on the contrary, showed that in his case the network had apparently acted as a trap which ensnared the embolus entering the auricle through the inferior cava, thus preventing a possible fatal pulmonary embolism.

CASE REPORTS

CASE 1. A woman, 67 years of age, on the service of Dr. G. H. Hoxie, gave a history of having had heart disease "almost all her life." During the latter part of her illness the patient developed auricular fibrillation and became quite dyspneic. An X-ray revealed what was thought to be fluid filling almost the entire left chest. The pleural cavity was aspirated and only blood was obtained. The patient subsequently died of cardiac failure.

The only autopsy findings of interest were those encountered in the chest. The left lung was markedly atelectatic, being compressed by the extreme dilatation of the left auricle. The lung was atrophied, so that it measured only 5 cm. in its greatest diameter. The left auricle was extremely dilated, and had a 1000 cc. capacity. This finding might possibly account for the incorrect X-ray interpretation. There was a striking stenosis of the mitral valve with great

THE FREQUENCY OF ANOMALOUS RETICULA IN THE RIGHT ATRIUM OF THE HUMAN HEART "CHIARI NETWORK" *

REPORT OF EIGHT CASES

FERDINAND C. HELWIG, M.D.

(From the Department of Pathology, St. Luke's Hospital, Kansas City, Mo.)

In 1897 Chiari¹ reported eleven cases in which a fine network was found in the right cardiac atrium, and although it was first observed by von Rokitsky,² it has always been known as "Chiari's network." Anomalous variations of the venous valves of the right atrium are extremely common, and it is with these that Chiari's network might possibly be confused. A true "Chiari network" is an anomaly which is formed of strands and reticula which stretch from the thesbian and eustachian valves across the right auricle to insert either into the crista terminalis or the endocardium near the tubercle of Lower.

Several explanations of this anomaly have been proposed to account for its development. Chiari thought that it was remnant of the right venous valve of the septum spurium. Looser³ believed that it arose from a dislocation of the fibers from their normal points and took place as a result of irregularity in endocardial growth. Jordan,⁴ in reporting two cases, was inclined to the view that the network was formed from remnants of portions of the valves of the right horn of the sinus venosus. In a recent personal communication he is still of the same opinion.

Since Chiari's publication, fifteen additional cases have been studied and added to the literature. From the small number of reported instances of this anomaly one might assume that these structures are quite rare, while our findings lead us to believe the contrary is true. In a series of 460 autopsy examinations conducted at St. Luke's Hospital from January 1, 1928, to July 1, 1931, a period of three and a half years, seven hearts showing this anomaly were seen, an incidence of 1.5 per cent.

* Received for publication September 12, 1931.

At autopsy she had dependent edema, ascites, and a bilateral hydrothorax. In the brain an area of encephalomalacia was seen in the left lenticular nucleus. A large pulmonary infarct, with a thrombus occluding the branch of the pulmonary artery supplying the area of infarction, was also found. The lungs and liver showed considerable passive hyperemia. In the heart, which weighed 312 gm., the left auricle was widely dilated and the mitral valve was narrowed. The edges of the mitral leaflets were glued together by old, dense, calcified fibrous tissue, and there was marked calcification present on the inferior borders of the leaflets. In the appendage of the right auricle an adherent mural thrombus was present. The eustachian valve was fenestrated and a single round cord extended from the upper border of this valve across the right atrium and attached to the crista terminalis.

CASE 4. A man, 50 years of age, had for five years been having attacks of pain in the left chest which radiated down the left arm and localized in his elbow. These attacks were so severe that large doses of morphine were required for relief. During the last year he had been bedfast most of the time, having many attacks of tightness in his throat forcing him to struggle for sufficient air. On physical examination he had a "gallop rhythm" and an electrocardiographic tracing revealed a "right bundle branch block." While lying quietly in bed he had a sudden feeling of suffocating constriction in the throat, developed an apparent edema of the lungs, became markedly cyanotic, and died.

At autopsy the important findings were exclusively cardiac. The heart weighed 505 gm. Old fibrous adhesions were present between the epicardium and the parietal pericardium over an area of about 4 sq. cm. on the lateral surface of the left ventricle. All of the cardiac chambers were dilated, particularly the left ventricle, the wall of which was very thin, measuring no more than 4 mm. in thickness in the region of the interventricular septum. The endocardium covering the anterior two-thirds of the septum in the left ventricle and the anterior half of the ventricular wall was silvery white and thickened in character. The muscle beneath this was largely replaced by dense hyaline fibrous tissue. The descending branch of the left coronary artery was completely occluded and markedly calcified at a point 6 mm. from its ostium.

In the left auricle the thesbian valve was found to be nothing but two web-like, thin, filamentous bands which extended up and joined with a webbed eustachian valve. Here two delicate silk-like tendrils extended across the foramen ovale and attached to the tubercle of

shortening of the chordae tendineae, and, apparently, both stenosis and regurgitation were present. Both lungs showed edema and passive hyperemia. The remainder of the heart was not particularly hypertrophied. In the right auricle a typical "Chiari network" was found. The fibers extended from the thesbian and eustachian valves across a very large, closed foramen ovale (measuring 4 by 3 cm.), and were attached to the crista terminalis. The fibrillae, comprising this network, were thin and web-like in character. They met at a central point just over the foramen ovale, branching out and being attached at three different points along the crista terminalis at the opening of the superior vena cava.

CASE 2. This was a woman 43 years of age (a patient of Dr. F. C. Rumsey's), who had her first symptoms of cardiac failure in 1925, following unusual physical exertion. Except for rheumatic fever when a child, her cardiac history was negative. On her first admission to the hospital a diagnosis of mitral stenosis was made. She returned home, but on her second hospital admission she had a complete hemiplegia, doubtless of embolic origin. Several months later she had an occluding embolus of the left femoral artery which necessitated an amputation above the knee on account of dry gangrene. She gradually developed a loss of myocardial tone and died five years after her first heart attack.

At autopsy a large area of cystic softening was found in the right temporal lobe of the brain. The embolus which had caused the gangrene of the leg had become organized and existed as a knob-like cicatrix almost filling the arterial lumen. There was a marked sclerosing mitral and tricuspid stenosis with a bilateral atrial dilatation. The coronary sinus in the right auricle was considerably dilated, and the thesbian valve was fenestrated and extended across the coronary sinus as a web-like band reaching up to the eustachian valve, which was also fenestrated. From this point two thread-like cords crossed the atrium, joining at the point where they began to cross the foramen ovale, and extended as one cord to the crista terminalis, thus covering a distance of about 5 cm.

CASE 3. A woman, 38 years of age, entered the hospital complaining of "heart flutter and palpitation." She had had dyspnea on exertion for ten years. Her trouble, she thought, began twelve years ago, following influenza. A year ago she again had influenza and suffered considerably from heart trouble, with dyspnea and hemoptysis.

On physical examination there was a high pitched mitral murmur present which was transmitted over the entire left chest, and was heard best over the mitral and tricuspid areas. Two days before death she developed marked auricular fibrillation, and a right hemiplegia followed.

cord degeneration. The day prior to her death she began to bleed from the nose, and her tongue became very sore and greatly swollen. She died suddenly, apparently choking to death, the obstruction being due to the extreme swelling of her tongue. No abnormal findings or symptoms referable to the heart were ever elicited.

At autopsy the characteristic pathological anatomical alterations of pernicious anemia were encountered. The heart weighed 305 gm. The myocardium was extremely flabby, and showed the typical "tabby-cat" mottling of fatty degeneration. The valves were clean and smooth and the measurements within normal limits. The coronary arteries revealed a few fine intimal atheromatous plaques. The right atrium was of normal dimensions. The valve of the coronary sinus was composed of five white, fine reticular strands which did not connect with the eustachian valve. This latter valve was made up of six fine reticula which joined at a point about 1 cm. from their insertion on the inferior border of the inferior vena cava, and ran as a single cord for about 1 cm., where they again branched out into a very delicate web-like network of anastomosing fibrils which attached by nine insertions along the superior border of the inferior vena cava. From here, reticula of even finer and more delicate character stretched across the auricle to attach to the endocardium of the crista terminalis at ten different points. There was also a single band of considerably greater diameter than these previous threads which stretched downward from the lower border of the superior vena to attach to the endocardium in the region of the tubercle of Lower.

CASE 8. This case was discovered in a museum specimen and no history was obtainable. In this heart four different points of origin of the network were found; one on the lower rim of the coronary sinus, one from its upper rim, and two from the lower border of a vestigial fenestrated eustachian valve. These four cords joined almost immediately and extended across the auricle as two twisted threads to insert by one attachment to the crista terminalis.

SUMMARY AND CONCLUSIONS

In the first four cases of this series severe cardiac lesions were found which were the direct cause of the patient's death. In the last four cases the findings present in the heart, aside from the Chiari network, were negligible, and in none of the entire group could any of the clinical symptoms be ascribed to the network itself.

Lower. Two small delicate twiglets were also found which extended from the upper rim of the foramen ovale and attached to the endocardium just lateral to the tubercle of Lower. The aortic valve showed a sieve-like fenestration of two of its leaflets.

CASE 5. A young man, 21 years of age, died after having a spindle cell sarcoma removed from his right thigh, from a massive local recurrence with diffuse visceral sarcomatous dissemination. Physical examination revealed no evidence of cardiac trouble.

At autopsy emaciation was a striking feature, and, in addition to an enormous fungating growth on the right thigh, extensive metastases were found in the liver and lungs. The heart weighed 305 gm., was very pale, the muscle flabby, and the chambers moderately dilated. In the right auricle the eustachian valve was rudimentary, as was the thesbian valve over the coronary sinus. Communicating with the lower border of the eustachian valve and crossing the auricle over the foramen ovale was a single, rather tough, semiflattened band about 1.5 mm. in diameter, which was attached to the crista terminalis. At the point where it crossed the foramen ovale there was a fine branch which had broken off prior to the discovery of the structure, evidently having been torn loose at the time of opening the heart. This apparently was in communication with the eustachian valve at a point somewhat higher than the larger one.

CASE 6. This patient was a well nourished young man, 24 years of age, who entered the hospital semicomatose and irrational, giving a history of headaches for some time. He gradually became worse, and soon developed definite signs of an intracranial tumor. Physical examination on several admissions revealed no cardiac disturbance.

At autopsy a large infiltrating spongioblastoma arising from the cerebral peduncle was found, and the cardiac atrial anomaly was found during the routine examination. The heart weighed 210 gm., and all of the valve measurements were within normal limits. The chambers were filled with clots. In the right atrium two fine thread-like strands arose from the inferior border of the inferior vena cava and merged in the middle to form a small knob-like thickening, where they again forked and stretched up to the crista terminalis, there inserting by two attachments quite close together.

CASE 7. A short, fleshy, stocky woman, 53 years of age, had been treated for pernicious anemia for some time by the use of liver extract, and, although her anemia did not increase, she developed rapid and striking evidence of spinal

DESCRIPTION OF PLATES

PLATE 14

FIG. 1. Case 1. Showing Chiari network crossing right atrium. Note huge foramen ovali.

FIG. 2. Case 2. Dilated right auricle showing tricuspid stenosis, fenestrated eustachian valve and Chiari network.

Eight cases of Chiari's network are recorded, and, although so few cases have been reported, such reticula are not a particularly rare anomaly in the human heart, since they were found to be present in 1.5 per cent of routine autopsy examinations. These structures are easily destroyed by careless manipulation, and no doubt will be found quite often if more care is exercised in removing and opening the heart. They were of no clinical significance in this series of cases.

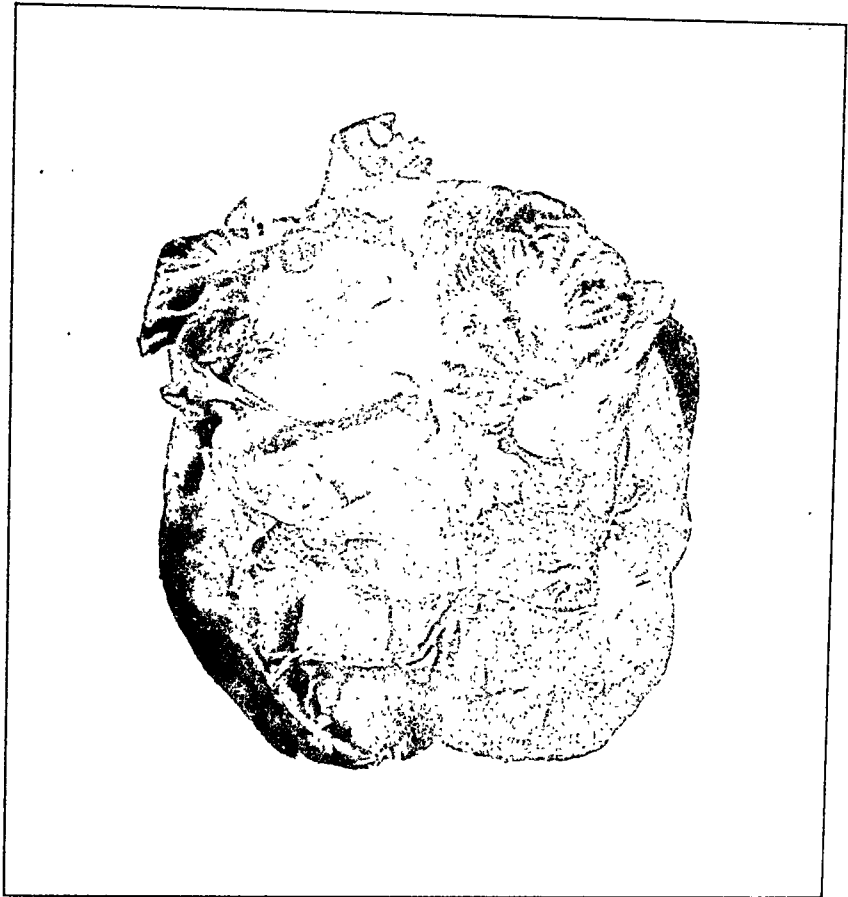
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PLATE 15

FIG. 3. Case 3. Right atrium showing fenestrated eustachian valve and Chiari network.

FIG. 4. Case 4. Right atrium showing marked fenestration of the eustachian valve with Chiari network inserting into the endocardium at two points.



1



2

PLATE 16

FIG. 5. Case 5. Right atrium showing Chiari network represented by a single flat band crossing the auricle.

FIG. 6. Case 6. Chiari network can be seen crossing the right atrium, showing knob-like thickening in the center.



3



4

Anomalous Reticula in Heart "Chiari Network"

PLATE 17

FIG. 7. Case 7. Right atrium showing markedly webbed Chiari network with numerous insertions.

FIG. 8. Case 8. Right atrium showing vestigial eustachian and thesbian valves with Chiari network extending across atrium to crista terminalis.



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Helwig

Anomalous Reticula in Heart "Chiari Network"





7



8

Helwig

Anomalous Reticula in Heart "Chiari' Network"

in which the existence of syphilis of the aorta could be regarded as certain. In neither of these was it likely that the valves were affected." He concluded that in only two cases out of thirty-five was there a serious possibility that syphilis might have been a predisposing agent in streptococcal endocarditis.

The following case represents an individual who suffered from syphilitic aortitis and aortic endocarditis (aortic insufficiency), and who, in the latter stages of his illness, contracted an aortic bacterial (*Streptococcus viridans*) endocarditis.

CASE REPORT

Clinical History: A colored hotel porter aged 23 years came to the accident room on Oct. 7, 1930, because of severe shortness of breath of thirty-six hours' duration. His father had died following a "stroke." His mother had died during the patient's childhood, leaving nine siblings living and well. The patient had measles, mumps, pertussis, and influenza as a child and youth. He had had frequent and severe sore throats until the age of 21. At 19 he had a chancre and received six injections of arsphenamine. Six months later he had a generalized skin rash and received six additional injections of arsphenamine. He had been perfectly well until three weeks before admission. At this time, while moving trunks, he had an attack of severe dyspnea, but without any precordial pain or other symptoms. After two days in bed because of the dyspnea, he returned to work feeling fairly well. He requested the less heavy work of the night porter, however. He then felt well until about thirty-six hours before admission. At that time he drank half a pint of whiskey to relieve a feeling of weakness before going to work. At 10 P.M., a few hours after beginning work, he became increasingly short of breath and was forced to quit. He was taken to a local hospital and was given some medicine, but was not admitted. That night and the following night he was unable to sleep because of the extreme dyspnea and orthopnea. Obtaining no relief from medicines, he came to the hospital the following day.

On admission to The Duke Hospital the temperature was 37° C., pulse 100, respirations 60, blood pressure 190/50. The patient was a pale, anxious, markedly orthopneic young negro whose head moved rhythmically with each pulse beat. There was no jaundice. Axillary and inguinal lymph nodes were palpable. Pupils were regular and equal and reacted to light and during accommodation. Fundi normal. No pulsations were seen. Pharynx and larynx were normal. The left chest was more prominent than the right and there was a visible precordial heave. The apex of the heart was under the sixth rib, 14 cm. to the left of the sternum. The first sound was accentuated and there was a very loud, rough, systolic murmur heard over the whole precordium. Over the aortic area and along the left border of the sternum there was a loud blowing murmur occupying all of diastole. There were numerous moist râles at both bases. The pulse was Corrigan in type. Pistol-shot sounds and capillary pulsations were obvious. The liver edge was palpable 2 cm. below the costal margin. The spleen was not palpable. There was slight pitting edema of the ankles.

SYPHILITIC AORTIC ENDOCARDITIS AND SUPERIMPOSED
BACTERIAL (STREPTOCOCCUS VIRIDANS)
ENDOCARDITIS *

E. B. CRAVEN, JR., M.D.

(From the Department of Pathology, Duke University School of Medicine,
Durham, North Carolina)

Bacterial lesions of the valves of the heart as a sequel to syphilitic changes are decidedly rare. Kastner¹ in 1918 presented a case of syphilitic aortitis and aortic insufficiency with the subsequent development of an ulcerative endocarditis (endocarditis lenta) of the aortic valve. The diagnosis was proved by autopsy. Briggs² in 1922 reported one case, with autopsy, of syphilitic aortic endocarditis and insufficiency, with the later development of *Streptococcus viridans* vegetations on the previously damaged valve. Curschmann³ in 1922 observed a case of clinical endocarditis lenta with aortic insufficiency, occurring in a patient with a positive blood Wassermann reaction. This patient had had typical rheumatic febrile attacks with polyarthritides. The diagnosis of rheumatic heart disease could not therefore be ruled out. There was no autopsy. Blumer⁴ in 1923 in a study of 330 cases of subacute bacterial endocarditis mentions syphilis as a predisposing cause in only one case. The details of this case are not given. Clawson⁵ in 1924 reported 220 cases of bacterial endocarditis in none of which were there syphilitic valvular lesions. Pineles⁶ reported four cases in 1926, but only one was studied at autopsy. Syphilitic aortitis and an ulcerative endocarditis (endocarditis lenta) were found. The offending bacterium was not identified by culture. The other three cases reported by Pineles were in individuals with positive blood Wassermann reactions and aortic insufficiency. From only one case was a bacterium (*Streptococcus viridans*) isolated. The occurrence of the combined lesion in the latter three cases is certainly not proved. The first case seems authentic. Thayer⁷ in 1926 in an exhaustive study of bacterial endocarditis "found among thirty-five instances of streptococcal endocarditis, acute and subacute, involving the aortic valves, but two

* Received for publication June 24, 1931.

Pericardial Cavity: No adhesions or excess fluid. The pericardial sac is almost completely filled by the enormously enlarged heart. Surfaces smooth and glistening.

Thymus: No thymus tissue seen.

Heart and Aorta: The heart is massively enlarged, weighing 630 gm. Measurements are as follows: Left ventricle 2 cm. in thickness, right ventricle 7 mm. in thickness, tricuspid valve 13.5 cm., pulmonary valve 9 cm., mitral valve 11.5 cm., and aortic valve 7 cm. There is an extreme dilatation of the left ventricle, as well as enormous hypertrophy, and the right ventricle is also markedly thickened. Epicardium smooth and shiny. The coronary vessels are patent and exhibit no atheromatous changes. The myocardium is of a homogeneous pale yellow color but shows no scars or infarcts. Except for the aortic valve (Fig. 1), the endocardium exhibits no lesions. The right cusp of the aortic valve is markedly retracted and shortened, the edge is thickened and grossly irregular, giving the appearance of having been bitten out. There is a perforation 2 mm. in diameter beneath its rolled edge. On this same cusp, near its attachment to the posterior cusp, there is a series of soft, friable, grayish pink vegetations which occupy an area 1.5 sq. mm. on the ventricular surface of the valve. From a portion taken for culture, *Streptococcus viridans* was identified, and the same organism was recovered from the blood stream. The posterior aortic cusp is definitely retracted and thickened, but the anterior cusp is essentially normal. The aortic leaflet of the mitral valve, over a small area exactly juxtaposed to the aortic vegetations, presents a few small protuberances. The mitral valve is otherwise normal, the edges thin and delicate. Chordae tendineae smooth, slender and delicate. Pulmonary and tricuspid valves show no abnormalities.

On the wall of the aorta, immediately above the attachment of the posterior cusp, there is a wrinkled patch 1 cm. in diameter. The intima here is thrown up into coarse folds and rugae. The cut surface shows the media interrupted by dense scars. The adventitia is conspicuously thickened. There is a similar small puckered area above the anterior cusp and another on the wall of the descending arch. Both show characteristic medial scars. The coronary orifices are not involved in the syphilitic process. Microscopically there is an extraordinary quantity of fat in the muscle fibers. In some places

Laboratory Findings: Hemoglobin 11.5 gm.; red blood cells 4,100,000; white blood cells 9,400. The differential count was essentially normal. Urine: acid, specific gravity 1021, sugar 0, albumin 2 plus, occasional white blood cell. Blood non-protein nitrogen 47 mg. per cent; uric acid 4.8 mg. per cent; creatinine 1.8 mg. per cent. Total proteins 6.17 gm. per cent. Albumin-globulin ratio 62:38. Wassermann reaction strongly positive. Electrocardiogram showed normal sinus rhythm, levogram predominant. X-ray of the chest showed the heart enlarged in all diameters. The arch was not increased in width.

The diagnosis was syphilis, aortic insufficiency, relative mitral insufficiency, cardiac hypertrophy and dilatation, cardiac decompensation. Two nights' rest and digitalization produced remarkable improvement. The patient felt so well that it was difficult to keep him in bed. X-ray of the chest after a week, when the patient insisted he was well, showed no change in the heart shadow. At the time of discharge, three weeks after entry, there were signs of cardiac enlargement and aortic regurgitation. The blood pressure was 148/62. He was able to walk about without any dyspnea. He was discharged on Nov. 1, 1930, having had an afebrile course throughout.

Six weeks after discharge one of the patient's friends telephoned that the patient had been drinking heavily and that he was then afflicted with severe dropsy. He was advised to return to the hospital, but nothing was heard from him until Jan. 10, 1931, when he was seen at the Lincoln Hospital. He was extremely orthopneic, pale, worried, weak and discouraged. There was massive edema of the extremities and scrotum. While at the Lincoln Hospital he received three 0.60 gm. doses of neoarsphenamine. On January 18 one quart of fluid was removed from the abdomen. On January 22 he was seen to be growing steadily weaker, and death occurred at 4.30 A.M. on January 23, seventeen weeks after the onset of acute symptoms.

AUTOPSY REPORT

External Appearance: The body is that of a young, adult negro male. There is marked pitting edema of all of the subcutaneous tissues. Skin smooth and glistening. No tissue jaundice. Hair distribution normal. Pupils equal. Nasal septum intact. Teeth in good repair. The scrotum is edematous, but its contents are normal.

Abdominal Cavity: Peritoneal surfaces smooth and glistening. There is no excess fluid. The liver projects 4 cm. below the costal margin. The tip of the spleen is barely visible below the costal margin. Intestines in normal position.

Left Pleural Cavity: No excess fluid. Surfaces smooth and glistening except for a small area of fibrous adhesions on the posterior surface of the upper lobe.

Right Pleural Cavity: There are 500 cc. of clear yellow fluid, but no adhesions. The pleural surfaces are smooth, but have lost their glistening appearance.

bodies and trabeculae, while visible, are not conspicuous. The organ as a whole is quite firm and elastic. There are two small accessory spleens. Microscopically, the vessels are distended with blood. There is an unusual amount of fibrous tissue in the pulp. Numerous clumps of iron pigment are noted.

Intestines: Normal.

Stomach and Duodenum: The gastric mucosa appears injected because of the marked chronic passive congestion. Duodenal mucosa normal. Ampulla of Vater patent.

Pancreas: Grossly and microscopically normal.

Liver: The organ weighs 2,000 gm. and measures 27 cm. by 22 cm. by 10 cm. Capsule thin and delicate. The cut surface discloses lobules with large dark red central zones and surrounding pale yellow liver tissue, the appearance being that of the typical "nut-meg" liver of chronic passive congestion. The gall-bladder and bile ducts are normal. Microscopically, there is an extreme chronic passive congestion. The central veins are dilated and the surrounding capillaries filled with blood. In the central two-thirds of the lobule there is hardly a recognizable liver cell. Around the portal spaces there is a narrow zone of normal liver cells.

Adrenals: The glands together weigh 30 gm. There are no gross or microscopic alterations.

Kidneys: The kidneys are alike and normal in size and shape. The left weighs 170, and the right 160 gm. The capsules are thin, delicate, and strip easily. The cortices are smooth and the cut surface shows them to be of normal thickness. Pyramids and pelves normal. Microscopically the cells of the convoluted tubules are excessively pale and swollen. The lumina are filled with albuminous material which is also conspicuous in the capsules of the glomeruli. The blood vessels are distended but are otherwise normal. No alterations of the glomerular tufts are seen. There is no blood in the tubules.

Bladder: Normal.

Prostate: Normal.

Seminal Vesicles: Normal.

Testis and Epididymis: Normal.

Neck Organs: The epiglottis and vocal cords present no lesions. There is a diphtheritic exudate on the surface of the trachea. Thyroid normal in size and consistency. Esophagus normal.

it is so pronounced as to impart to the muscle bundles a striking pallor and moth-eaten appearance. Specific fat stains show the droplets to be within the sarcolemma sheath. The various branches of the coronary arteries are normal. There are no scars or areas of leucocytic infiltration in the myocardium. The section of the aortic valve (Fig. 3) shows an extreme thickening, scarring, and vascularization. There are great numbers of mononuclear cells, for the most part lymphocytes, about the blood vessels. In addition to the chronic changes, there is a fresh fibrinous exudate on the surface of the valve. Caught in the meshes of fibrin are polymorphonuclear and mononuclear leucocytes. The Gram-Weigert stain shows a small number of Gram-positive cocci occurring singly and in pairs. There are no bacteria in the thickened and scarred valvular tissue. The section from the aortic surface of the mitral valve shows a fresh fibrinous exudate like the above. There are a few Gram-positive cocci present, singly and in pairs. The lesion here seems to be clearly the result of contact with the infected aortic cusp. The adventitia of the aorta is thickened by an excess of fibrous tissue, and the blood vessels are surrounded by dense mantles of lymphocytes. The media of the vessel is repeatedly interrupted by large dense scars. An unusual number of blood vessels are present in such areas, all with perivascular lymphocytic infiltration. The Verhoff-Van Gieson stain shows interruption and fragmentation of the elastic tissue fibers (Fig. 2). The scars extend well into the inner third of the media.

Lungs: Both lungs are dark red in color and have a conspicuous rubbery consistency. The lower half of the right lower lobe is compressed. The cut surfaces disclose a few small grayish patches in the left lower lobe which contain no air. Microscopically, there are small patchy areas of pneumonia. The alveolar exudate is fairly fresh, being made up of well preserved leucocytes, fibrin strands, and a few red blood cells. The most striking lesion consists of the overfilling and distention of the capillaries with blood and the spilling of blood into the alveoli. There are great numbers of large mononuclear cells filled with iron pigment. Some of the pigment appears to lie free in the tissues of the alveolar walls. There are Gram-positive cocci in the areas of consolidation.

Spleen: The organ weighs 120 gm. The capsule is thin and stretched, cut surface flat, pulp dark red in color. The malpighian

1. A negative history of articular rheumatism.
2. The history of primary and secondary syphilitic lesions.
3. A strongly positive blood Wassermann reaction.
4. Absence of lesions of rheumatic fever in the pericardium, myocardium, and mitral valve.
5. The characteristic syphilitic retraction, perforation and serrated edge of the aortic valve.
6. Large and prominent perivascular infiltrations of the aortic adventitia, composed almost exclusively of small lymphocytes.
7. Large aortic vascular medial scars penetrating deeply into the medial coat, and with the formation of new vascular channels.
8. The acute exudate composed of fibrin, leucocytes and Gram-positive cocci (*Streptococcus viridans* as shown by culture) on the surface of the aortic valve.
9. The identification of *Streptococcus viridans* not only in a culture of the aortic valve vegetations, but also from the blood.

Retroperitoneal and Thoracic Tissues: The aorta is described above. The mesenteric, renal, and coeliac arteries are not thickened. Portal veins and venae cavae patent. Lymph nodes not enlarged.

Skeletal Tissues: The bones and joints appear normal on external examination.

Blood-Forming Tissues: The bone marrow of the femur is made up entirely of fat. That of the vertebrae is rich in blood-forming cells.

The axillary and inguinal lymph nodes are enlarged.

Anatomical Diagnoses: Syphilis, syphilitic aortitis, syphilitic aortic endocarditis and aortic insufficiency; fresh *Streptococcus viridans* aortic endocarditis; dilatation and hypertrophy of heart; extraordinary fat accumulation in heart muscle; chronic passive congestion of lungs and abdominal viscera; anasarca; right hydrothorax; lobular pneumonia; accessory spleens; tracheitis.

DISCUSSION

The foregoing report is presented as a case of syphilitic aortitis and aortic endocarditis with a superimposed *Streptococcus viridans* endocarditis. It should be kept in mind, however, that Klotz,⁸ in 1912, and more recently, Pappenheimer and VonGlahn⁹ in 1924, described lesions of the aorta occurring in non-syphilitic individuals with rheumatic heart disease, which might be confused with syphilitic aortitis. The lesions they described consisted of dense scars in the vicinity of the nutrient vessels, often acellular, and Aschoff cells or nodules in the adventitia, with large mononuclear and multinuclear giant cells. Other distinctive features of the rheumatic aortic lesions were the relative avascularity of the medial scars and the lack of penetration of these scars into the inner third of the media. In the adventitial nodules there were comparatively few lymphoid cells and never the dense perivascular lymphoid infiltrations customarily seen in syphilis.

SUMMARY

In summary, the opinion that in the case reported here the changes in the aorta and aortic valve are basically syphilitic in nature, with a superimposed bacterial endocarditis, is supported by the following facts:

DESCRIPTION OF PLATE

PLATE 18

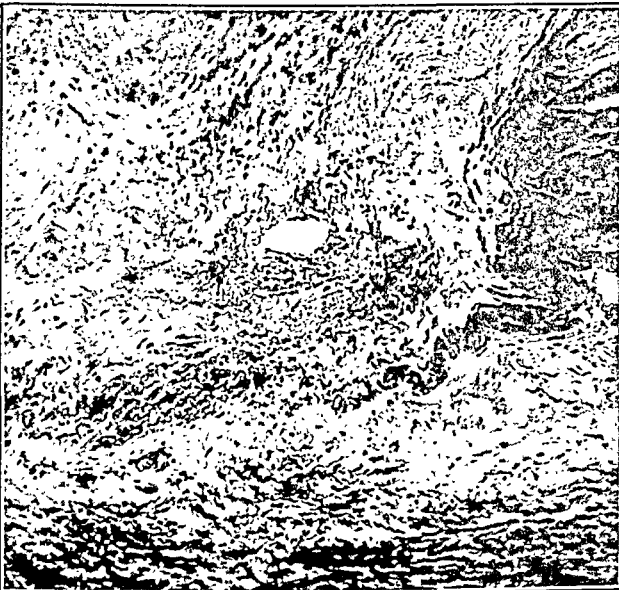
- FIG. 1. Aorta and aortic valve. The right coronary cusp is thickened and retracted and shows a serrated and perforated edge. The posterior cusp is thickened and retracted. Above the attachment of the posterior cusp there is a wrinkled, puckered patch of syphilitic aortitis.
- FIG. 2. Photomicrograph of the medial coat of the aorta. The interruption and fragmentation of the elastic fibers and the large medial scar are characteristic of the lesions found in the aorta. Verhoff-Van Gieson stain. $\times 150$.
- FIG. 3. Photomicrograph of the aortic valve. The valve is thickened and infiltrated by mononuclear cells. Attached to the surface is a fresh fibrinous exudate, in the meshes of which are groups of leucocytes. Hematoxylin and eosin stain. $\times 150$.

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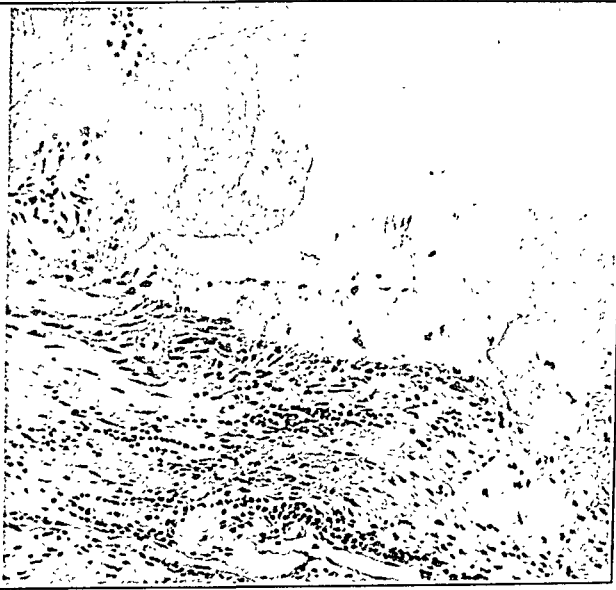


I



2

Craven



3

Syphilitic Aortic Endocarditis

Under these conditions the measurements of the photographic image of a valve bear the same relationship to the actual measurements as the measurements of the photographic image of the metal square bear to its actual measurements. The photograph was usually slightly larger than natural size.

The surface areas of the valve leaflets were determined by means

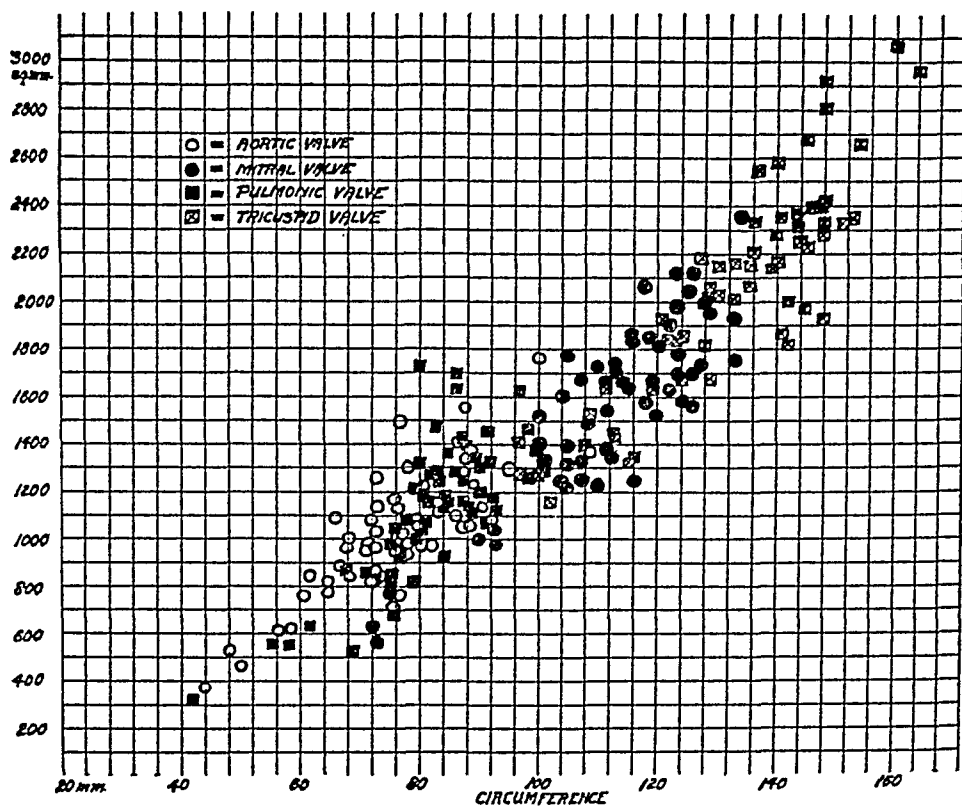


Chart I.

of a planimeter on photographic prints. At least two determinations were made with a difference of less than 3 per cent.

The majority of the hearts in this series were photographed within thirty-six hours after death, although there are several hearts which were photographed as late as seventy-two hours postmortem. A subsequent investigation has proved that variations due to post-mortem changes are of little consequence.

QUANTITATIVE OBSERVATIONS ON THE VALVES OF THE HUMAN HEART *

PAUL GROSS, M.D., AND ROBERT A. MOORE, M.D.†

(From the Pathological Laboratories, Cleveland City Hospital and The Institute of Pathology, Western Reserve University, Cleveland, Ohio)

For many years pathologists have measured the circumference of the valve rings of the heart, and some have attempted to interpret such measurements for or against the existence of incompetency of the valve orifice without regard for the amount of valve surface. It is obvious however that ring circumference is only one factor and that the area of the leaflets must be correlated with the circumference in order to obtain a true judgment. During the course of postmortem examinations it is frequently noted that a heart with large valve rings has large valve surfaces. The fact that the valve surfaces of an adult heart are larger than those of a child's heart is obvious and need not be stressed. By the method to be described, the valve surfaces were measured in an attempt to correlate these variations in valve surface with the weight or age of the heart. Although this proved unsuccessful, a very significant relationship between ring circumference and valve area was found. In this paper we wish to present the results of the measurements of the valves of sixty-four hearts.

METHOD

The method is essentially one of computing lengths and areas from photographs of known magnification. The photographs are prepared as follows: The valves of human hearts removed at autopsy are opened in the usual manner and pinned upon a soft board in such a way that the valve ring is stretched under a tension sufficient to pull it into a flat plane without distortion. The valve leaflets or cusps are transfixed by pins so that their entire surface is presented to the camera. A metal square of known dimension is placed beside and in the same plane as the preparation (Fig. 1).

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† Hanna Research Fellow in Pathology.

fact serves as a support for the assumption that the increase in valve surface is gradual and is coincident with the increase in the circumference of the valve ring.

SUMMARY

1. A method for the determination of the surface area of valves is described.

2. The surface area of a valve with a large ring is commensurately greater than that of a valve with a normal ring.

TABLE I
Effect of Time on the Measurements of Heart Valves

Aortic valve

Hours Postmortem	Circumference	Valve surface	Difference
	<i>mm.</i>	<i>sq. mm.</i>	<i>per cent</i>
4.....	62	803	...
150.....	62	840	4.6

Pulmonic valve

4.....	81	1070	...
150.....	79	1030	3.7

Mitral valve

4.....	108	1260	...
150.....	110	1220	3.2

Tricuspid valve

4.....	143	2160	...
150.....	142	2000	7.4

RESULTS

The experimental error was estimated by photographing the same valve at different magnifications and making several prints of one negative. Subsequent calculations from these prints yielded results which did not vary more than 5 to 7 per cent. Comparisons between circumferences of valve rings as obtained from the pictures and those obtained by actual measurements resulted in differences of 3 to 7 per cent.

The effect of time on the measurements of the valves is illustrated in Table I (Case No. 6356). The results indicate differences between 3.2 and 7.4 per cent which may be considered within the experimental error.

Sixty-four hearts were photographed. These photographs consist of fifty-five aortic valves, fifty-six pulmonic valves, fifty-seven mitral valves, and fifty-eight tricuspid valves. The measurements of these valves are listed in Table II. A graphical representation of this table is given in Chart 1. This graph indicates that there is a commensurate increase in valve surface with an increase in the circumference of the valve ring. The rate of increase of valve surface is approximately the same for all four valves.

DISCUSSION

The majority of the hearts are from patients who suffered from coronary sclerosis, generalized arteriosclerosis, pulmonary tuberculosis, luetic aortitis, or pulmonary emphysema. The ages vary from 4 years to 80 years. Because of the variability in age and disease, the circumferences of the valve rings cannot be correlated with the age or the weights of the hearts. In all cases except those noted, the valve leaflets were considered normal.

In this series of hearts, many valves were encountered whose rings can be definitely termed dilated. Yet these valves have correspondingly large surfaces. Such large valve surfaces as these are not found with small rings. It therefore appears probable that the increase in valve surface, whether due to stretching or to actual growth, is a gradual process which keeps pace with the dilatation of the ring. The valves of young normal hearts coincide accurately with the downward projection of the curve of large valves. This

DESCRIPTION OF PLATE

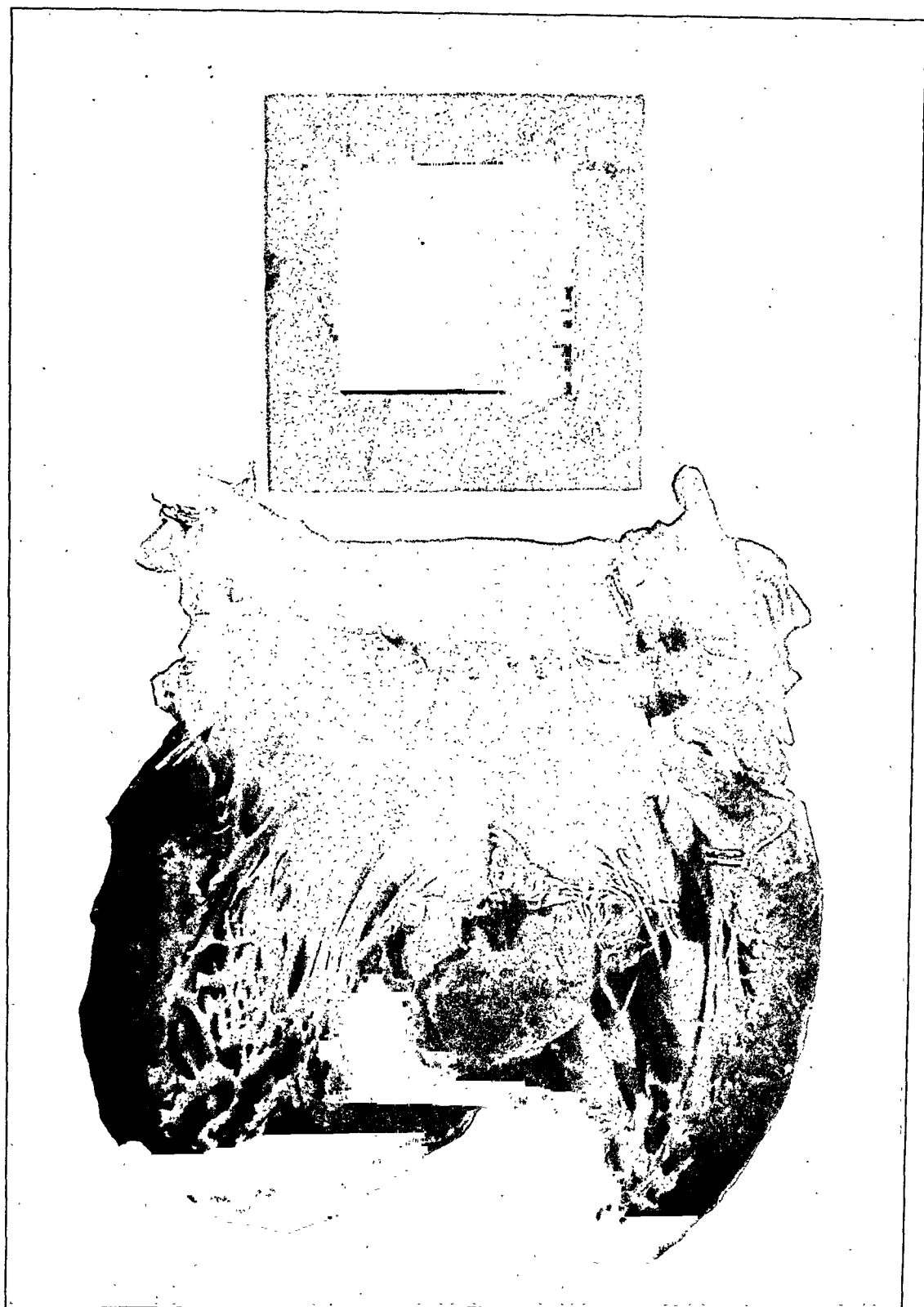
PLATE 19

FIG. 1. Type of photograph used for measurement of valves.

TABLE II
Data and Measurements of Heart Valves

Case No.	Weight	Age	Aortic valve		Pulmonic valve		Mitral valve		Tricuspid valve	
			Circum- ference	Valve surface	Circum- ference	Valve surface	Circum- ference	Valve surface	Circum- ference	Valve surface
			mm.	sq. mm.	mm.	sq. mm.	mm.	sq. mm.	mm.	sq. mm.
6212	225	36	72	1075	89	1350	105	1220	123	1840
6220	300	38	79	1050	84	1130	119	1530	136	2340
6222	525	37	75	1160	89	1108	105	1780	129	2014
6223	400	38	77	766	85	932	89	1220	112	1450
6227	350	65	92	1090	89	1130	116	1255	135	2078
6228	600	59	82	1260
6230a	425	60	87	1340	90	1200	127	1740	148	2240
6230b	79	1090	86	1640	113	1740
6233	350	54	78	1300	91	1320	122	1640	136	2200
6234	200	70	68	1000	80	1730	105	1400	129	2050
6235	250	22	56	610	76	680	93	990	108	1400
6236	325	30	87	1060	93	1110	107	1690	130	2150
6237	280	24	68	850	77	930	111	1540	128	1810
6238	250	23	61	770	72	810	105	1310	122	1830
6239	400	62	83	1150	84	1140	114	1670	139	2140
6240	175	37	58	610	71	860	93	1020	115	1310
6243	570	56	83	1120	87	1400	126	1570	130	2020
6247	430	28	66	1100	97	1630	119	1670	148	2290
6250	210	21	65	790	73	830	100	1400	116	1330
6251	350	75	81	1220	92	1170	*	...	127	2190
6254	400	54	75	700	75	990	105	1220	111	1620
6256	175	47	90	1140	80	1030	111	1390	119	1660
6257	275	54	73	850	76	1050	118	1580	148	1930
6259	100	7	48	530	55	560	72	630	109	1100
6261	150	22	58	810	78	690	99	1140
6262	275	70	87	1140	145	1970
6263	500	57	76*	950	79	1000	*	...	142	1810
6268	625	57	87	1290	85	1360	123	1690	148	2420
6284	250	63	100	1780	86	1700	118	2080	145	2700
6285	325	47	76	980	82	1270	126	1700	135	2250
6289	425	76	88	1380	83	1470	115	1650	133	2170
6290	450	30	72	960	101	1340	113	1420
6291	300	44	65	810	62	630	107	1260	100	1280
6299	425	60	*	...	81	1180	116	1820	145	2230
6328	600	38	81	1190	128	2000	141	2370
6339	600	32	73	1130	110	1720	151	2320
6342	400	63	88	1050	84	1190	133	1760	144	2230
6349	460	80	78	930	107	1320
6350	800	31	77	1030	68	870	109	1510	129	1690
6355	350	58	82	970	87	1250	122	1900	143	2380
6356	225	31	62	840	81	1070	110	1220	142	2000
6358	600	27	68	970	86	1280	124	1580	140	2170
6382	500	53	73	1040	78	1090	113	1720	147	2400
6384	700	59	*	133	1940	154	2360
6403	450	39	76	1140	90	1300	112	1380	141	1870
6405	625	52	109	1370	101	1290	120	1810	140	2290
6416	300	42	90	1000	97	1290
6419	625	56	86	1100	83	1250	118	1830	148	2810
6421	400	59	76	1010	91	1090	118	1690	133	2020
6424	250	41	73	841	75	800	104	1600	124	1860
6432	575	50	80	980	79	1210	*	...	143	2320
6496	800—	70	*	...	91	1460	116	1810	160	3080
6509	700	59	87	1560	80	1330	123	1970	148	2940
6524	800	66	95	1300	98	1460	125	2040	137	2550
6528	...	4	123	2110	140	2590
6529	350	38	77*	1500	97	1410	134*	2370	154*	2660
6531	800	40	71	945	123	1780	121	1920
6532	700	40	87	1410	98	1260	129	2070	146	2400
6534	275	26	100	1520
6547	700	34	73	1260	99	1380	126	2110	164	2960
6554	125	7	50	460	58	550	75	785	102	1160
6736	300	30	71	970	78	816

* These valves were the seat of an acute or chronic endocarditis with a variable amount of functional change.



I

Gross and Moore

Quantitative Observations on Valves of Heart

originating from undifferentiated lymphoid cells which circulate in the blood; later the newly formed myeloid elements pass through the wall of the vessel, particularly when the blood stream is slowed, and enter into the tissue in which proliferation is continued; (2) in lymphoid structures, including the spleen, free basophilic tissue cells and lymphocytes may be the source of myeloid elements; such a process has been found in the germinal centers of such structures, and (3) early myelocytes may appear outside the endothelium of small blood vessels and originate directly from fixed elements of undifferentiated embryonic character; these cells divide mitotically, become isolated, and skipping the stage of basophilic hemocytoblast immediately begin to develop specific granules in their protoplasm.

In the embryo the hemocytoblasts of the bone marrow originate from the fixed and undifferentiated mesenchymal cells. In the adult it is rare to have this process occur, because mitoses of preëxistent hemocytoblasts are sufficient to supply the needs of the body for new myeloid elements. However, under the stimulus of disease new formation of hemocytoblasts from the undifferentiated syncytium may occur.^{1, 2}

Arnold,³ in 1866, in a study of the cytology and the chemical reactions of the suprarenal glands mentioned for the first time that these glands might develop blood cell elements from ectopic islands of bone marrow within their confines. May,⁴ in 1887, reviewed the pathological anatomy of forty-two suprarenal glands, but did not mention the presence of bone marrow. Arnold,⁵ in 1896, reaffirmed his belief concerning the ability of islands of bone marrow in the suprarenal glands to form hemoglobin, and cited a case. Sacerdotti and Frattin,⁶ in 1902, studied the formation of hyperplastic bone marrow and stated that bone marrow is occasionally found in the suprarenal glands.

Gierke,⁷ in 1905, was the first to present a description of the histological data concerning bone marrow found in the suprarenal glands. Similar observations were reported by others (Tables I and II).

Tanaka,⁸ in 1912, reported finding bone marrow at the hilum of the left kidney of a boy, aged $1\frac{1}{4}$ years, who died from rickets and bronchopneumonia. Certain authors stated that heterotopias of bone marrow that occur in the suprarenal glands represent premyelomas. Oberling⁹ designated ectopia by the term "myelolipoma." Moretti¹⁰ stated that masses of cells that appear to be lymphocytic

FORMATION OF BONE MARROW IN THE SUPRARENAL GLAND*

DONALD C. COLLINS, M.D.

FELLOW IN PATHOLOGY, THE MAYO FOUNDATION

(From the Section on Pathologic Anatomy, The Mayo Clinic, Rochester, Minn.)

The formation of bone marrow in the human body in sites other than the bone is frequently observed in old age, particularly in the presence of ossification of the laryngeal cartilages. Bone marrow has been reported to form in the spleen, liver, sclerotic aortic wall, and sometimes in the suprarenal glands. Experimentally, it has been found in kidneys of adult rabbits following ligation of the renal artery and vein.

Leukemia, some intoxications and infections with experimental causes such as hemorrhage, or chronic poisoning by certain substances that destroy the blood, are well known agents which influence the organism as a whole and cause the formation of extramedullary myelopoiesis in various parts of the body. The spleen is usually the first to be affected, followed later by the liver, lymph nodes and suprarenal glands. Early in most cases neutrophilic and eosinophilic myelocytes appear in the new situation, followed later by megakaryocytes and finally by erythroblasts, although these are not common. Histologically, extramedullary hemopoiesis is of considerable significance in studying the histogenesis and the interrelations existing between the myeloid and lymphoid elements of the blood. Maximow and Bloom¹ stated that the fixed histocytes are believed to be the stem cells and may be endowed with unrestricted mesenchymal potency. These stem cells divide and give origin to free hemocytoblasts, which in their turn proliferate and differentiate into erythrocytes and myelocytes. Occasionally the endothelium of the blood vessels may give origin to myeloid elements, and from this point of view may possess considerable hemopoietic tendencies. Maximow and Bloom stated the belief that extramedullary myelopoiesis may originate in one of the following ways: (1) the first myeloid elements may appear in the lumen of venous capillaries,

* Received for publication July 13, 1931.

TABLE I

Cases of Bone and Bone Marrow in the Suprarenal Glands Reported in the Literature

Year	Author	Age	Sex	Side involved	Size in mm.	Autopsy data
1866	Arnold			Right		
1905	Gierke		M	Left	24 × 12.5	Mitral insufficiency and stenosis, old mitral and recent aortic endocarditis
1906	Brian	62	F	Left (para)		Carcinoma of fundus of uterus, regional metastasis
1911	Hirschfeld		M	Left		Hemicephalus in a new-born infant
1913	Hopf					
1916	Wooley	42	M	Left	6 × 3 1 × 0.5	General miliary tuberculosis, tuberculous costochondritis, acute vegetative aortic endocarditis, caseous tuberculosis of right suprarenal gland and kidney
1919	Mieremet	53	M	Left	60 × 35 × 12	Carcinoma of esophagus, aspiration bronchopneumonia
1922	Herzenberg			Accessory suprarenal	1 × 2	Generalized arteriosclerosis, aneurysm of left ventricle, atrophy of liver and spleen, cardiac hypertrophy
1922	Dieckmann		M	Left		
1925	Jedlička					Two cases
1927	Vigi	70	M	Right		
1928	Knabe	75	F	Right	7 × 6	Generalized arteriosclerosis
1928	Omelskyj	67	F	Left	8 × 4.5	Tuberculous pericarditis, mediastinal lymphadenopathy
1929	Paul	54	F	Left		Confluent bronchopneumonia, advanced generalized arteriosclerosis and coronary sclerosis, arteriosclerotic atrophy of kidneys, marked internal hydrocephalus
1930	Soós	58	M	Left	16 × 15	Hemorrhage into the pons of the brain

are not normal histological components of the suprarenal glands. Goldzieher¹¹ contended that bone, osteomas and bone marrow occurring in the suprarenal glands are the result of a metaplasia of inflammatory or scar tissue and that it is comparable to the formation of bone in burned-out foci of tuberculosis found elsewhere in the body. It is his belief that more or less extensive foci of adipose connective tissue intermingled with myeloid elements that occur in the suprarenal glands and present the appearance of bone marrow should be considered as belonging to that group of conditions characterized by foci of lymphocytes, as seen in association with granulomas of congenital syphilis, tuberculosis, and so forth. He stated that these areas of pseudobone marrow may be the direct result of a specific infectious process.

It is generally believed that the origin of bone marrow in the suprarenal glands is embryonal,^{7, 12-20} but certain authors²¹⁻²⁴ believe that bone marrow in the suprarenal glands arises from either capsular or cortical rests. Paunz²⁵ stated that bone marrow in the suprarenal glands arises from preëxisting bone marrow found there at the time of birth. Wooley²⁶ is inclined toward the hypothesis that bone marrow arises in the suprarenal glands from metaplasia of preëxisting suprarenal cells. He stated that formation of bone commonly occurs following trauma, with which there has been free extravasation of blood into the stroma of the traumatized tissue. He quoted Poscharissky, who believes that there is a peculiar disposition in different organs of the body toward ossification, and that in some organs the degree of congestion must be much greater before ossification occurs. It is well known from experimental work that more than 50 per cent of kidneys ossify following ligation of their blood vessels. Kruse,¹⁷ and Newsam¹⁸ stated that the calcium occurring in their cases was due to embryonal metaplasia, and that a specific toxin caused necrosis which was subsequently followed by calcification. Kovács,²⁷ in 1928, reported an exhaustive study on the pathological anatomy of the suprarenal glands in which formation of bone marrow was mentioned. Paunz²⁵ contributed a histological study on the cells that appeared to be lymphocytic in the suprarenal glands. He studied exhaustively the functions of the macrophages, but did not report instances in which bone marrow was encountered.

Soós¹⁶ classified cases of bone marrow occurring in suprarenal glands (Table II) into two types. The first type is characterized by a yellowish orange color, and on microscopic examination is seen to have a predominance of adipose connective tissue. The myeloid elements are diminished and the erythroblastic elements are increased. The second type is identified by dark red to reddish brown nodules, which on microscopic examination reveal a predominance of the cellular elements in the bone marrow, and in which the myeloid elements are increased and the erythroblastic components diminished (Table II).

In a case of bone marrow in the right suprarenal gland, observed at The Mayo Clinic, the patient was a man aged 32 years. He died of a totally unrelated disease. Gross examination disclosed a reddish brown nodule at the upper pole of the right suprarenal gland, measuring 12 by 9 by 8 mm. It was surrounded by a thin layer of normal appearing cortical suprarenal tissue averaging 1 mm. in thickness. The dimensions of the gland were 40 by 25 by 10 mm. The left suprarenal gland, aside from two cortical adenomas, each 1.5 mm. in diameter, appeared to be normal both grossly and microscopically. Its dimensions were 50 by 30 by 10 mm. The gross appearance of the upper pole of the right suprarenal gland is shown in Fig. 1.

Fixed sections 6 microns in thickness were stained by hematoxylin and eosin, and Giemsa-Wright stain. Five sections taken at different levels through the nodule revealed the following. With low power magnification the nodule was shown to be composed of considerable adipose connective tissue in which were islands of myeloid tissue. The nodule was entirely surrounded by normal appearing cortical cells. The arrangement of the tissue in the nodule was characteristic of bone marrow. Under high power magnification there was a comparative paucity of erythroblastic elements; only a few normoblasts were seen in the several sections. There were more lymphocytes than are usually seen in typical bone marrow. Myeloid regeneration was marked and numerous premyelocytes, as well as many polymorphonuclear forms, were seen. Megakaryocytes were prominent in the various sections. Aside from the slight degree of regeneration of erythroblasts and the increased numbers of lymphocytes in the nodule, it was composed of characteristic bone marrow. Figs. 2 and 3 show its microscopic appearance under different mag-

TABLE II

Classification of Cases Recorded in the Literature: So6s' Method
(Type 1)

Author	Gross appearance *	Bone marrow elements
Hirschfeld	Fatty +++-	
Hopf	Fatty +++-	Few myeloblasts, myelocytes, erythroblasts
Knabe	Fatty ++--	Erythroblasts, erythrocytes, myeloblasts, myelocytes, few macrophages
So6s	Fatty ++--	Erythroblasts, erythrocytes, myeloblasts

(Type 2)

Wooley	Cellular +---	Erythroblasts, erythrocytes, myeloblasts, myelocytes, lymphoblasts, lymphocytes, megakaryocytes (few)
Mieremet	Cellular +---	Erythroblasts, erythrocytes, myelocytes, polymorphonuclears, megakaryocytes, lymphocytes
Herzenberg	Cellular +---	Myeloblasts, erythroblasts, megakaryocytes
Brian	Cellular +---	Erythroblasts, erythrocytes, myelocytes, polymorphonuclears, few lymphocytes
Omelskyj	Cellular +---	Myeloblasts, myelocytes, polymorphonuclears, erythroblasts, macrophages, lymphocytes
Dieckmann	Cellular +---	Myeloblasts, myelocytes, polymorphonuclears, erythroblasts, lymphocytes
Gierke	Cellular +---	Myeloblasts, myelocytes, few polymorphonuclears and erythroblasts, lymphocytes
Paul	Cellular ----	Erythroblasts, erythrocytes, myelocytes, myeloblasts, megakaryocytes, granulocytes

* Ratio between fat cells indicated by + and bone marrow indicated by -.

The age of the youngest patient whose case was reported in the literature was 42 years, and the average age was 60.1 years (excluding Hirschfeld's case,²⁸ which occurred in a new-born infant). It was noted that only two patients died from symptoms and signs of suprarenal insufficiency; eight died from totally unrelated diseases. The average area of the bone marrow in eight reported cases was 15.4 by 9.9 mm.

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nifications. This case would seem to correspond to that of Soós' second type.

Since the suprarenal cortex and bone marrow both originate from mesenchymal tissue, it would be possible, under optimal physiological conditions with the proper stimulus, for cells of the suprarenal cortex to differentiate into bone marrow either during embryological development or possibly later. Embryonic rests of bone marrow are not commonly seen in the suprarenal glands. Embolism of bone marrow does not frequently establish new hemopoietic centers in distant histological structures.

SUMMARY

1. Fifteen instances of formation of bone marrow in the suprarenal gland have been collected from the literature, with a compilation of the salient features.

2. They have been classified according to Soós' method.

3. Theories concerning the mechanism of their origin have been discussed.

4. An additional example has been reported from The Mayo Clinic with illustrations of the salient gross and microscopic appearances.

DESCRIPTION OF PLATE

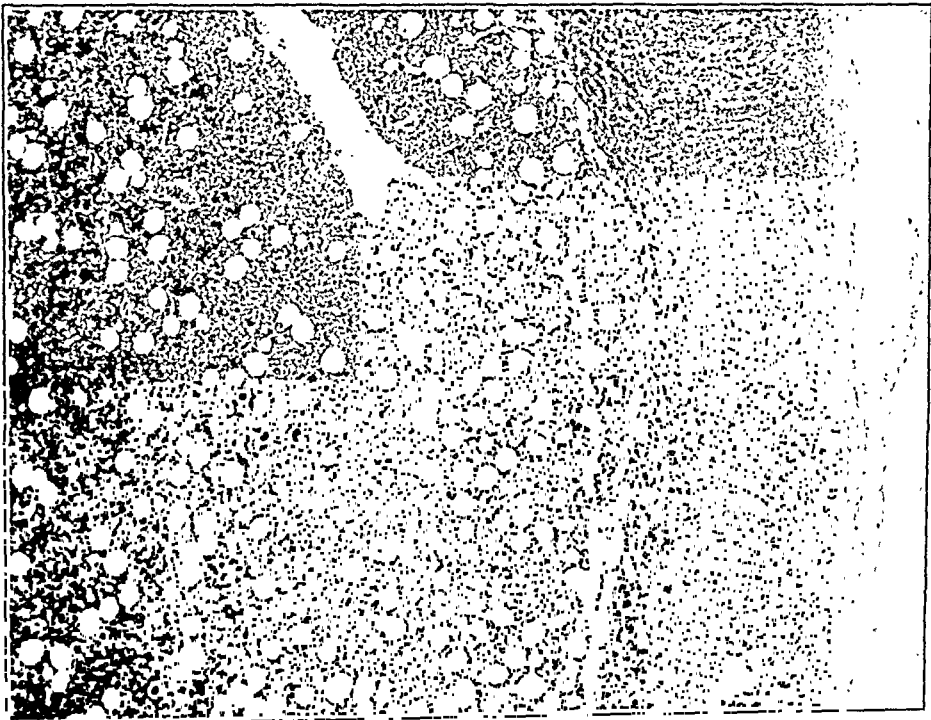
PLATE 20

- FIG. 1. Gross appearance of the upper pole of the right suprarenal gland.
- FIG. 2. Juncture between cortical suprarenal cells and typical bone marrow.
× 45.
- FIG. 3. Representative area in the bone marrow showing typical megakaryocytes, myeloblastic and erythroblastic elements. × 400.

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1



2



3

leaving an uneven granular and reddish gray surface. On section both cortex and medulla were reduced, the cortical striations were greatly distorted and the smaller vessels slightly prominent. The spleen was enlarged and weighed 140 gm., the capsule was thickened and stretched. On section, in the brownish red pulp, quite apart from the clearly defined follicles could be seen yellow and white areas of irregular outline. The trabecular veins were dilated and in many cases contained thrombi.

Microscopically only the kidney and the spleen were examined. In the spleen it is seen that the whitish areas represent necrotic splenic pulp in which the outlines of the tissue elements can still be recognized. The surrounding normal splenic pulp stands out in sharp contrast to the necrotic areas which are for the most part fresh, contain pyknotic nuclear debris, and only occasionally show early organization around the margin. The majority of these necroses show the zones which are usually found in infarcts, but a certain number are observed in which no such zones can be seen. These latter correspond to the atypical necroses described by Feitis, but a differentiation into types would appear to be artificial rather than real, since, although the extremes differ markedly, every stage between the two can be found. The pulp is normal; in places filled with blood both within and without the sinuses, in other places empty and collapsed. The follicles are greatly decreased in size and some indeed have almost disappeared. Often they are more apparent within the necroses than in the normal splenic pulp, for the reason that they are more resistant to the disintegration than the surrounding tissues. Kaufmann⁷ has observed that the follicles are the structures to remain visible longest in infarcts of the spleen. There is nothing, however, to support the observation made by Feitis that the degree of disappearance of the follicle coincides with the degree of change of the central follicular artery.

Most conspicuous changes are to be observed in the blood vessels. As other observers have noted, these changes differ with the size of the vessel. In the largest arteries, which run in the trabeculae, the intima is slightly proliferated and the lining endothelium occasionally swollen and vacuolated. In some cases there is an infiltration of leucocytes within the deeper layers of the intima. With an elastic tissue stain it is seen that the internal lamella is usually normal, but may be split to form two or three layers. The media is perhaps

MULTIPLE INFARCTS AND NECROSES OF THE SPLEEN (FLECKMILZ) *

GEOFFREY RAKE, M.B., B.S.

(*From the Pathological Institute, Heidelberg*)

The recognition of the peculiar condition in the spleen which has been given the name of "Fleckmilz" came from Feitis¹ who, in 1921, described two cases and suggested the name. That the condition is of some rarity is shown by the fact that up to the present time only eight cases,^{1, 2, 3} which can be accepted without demur as belonging to the group which Feitis described, have appeared in the literature.† The cases described by Geipel,⁴ Matthias,⁵ and certainly that by Wilton⁶ are to be considered as of a different category. The opportunity arose to study two cases of this condition in the Pathological Institute at Heidelberg, and since Fleckmilz has up to the present time received but little consideration in any but the German literature, and moreover since no complete understanding as to the pathogenesis of the condition has been reached, it was considered that a discussion of these cases might be of value.

DISCUSSION OF CASES

CASE I. (From the protocol.) Female, aged 44 years, who died in uremic coma. At autopsy the major changes were in the heart, kidneys and spleen. The wall of the left ventricle was markedly hypertrophied. The kidneys were alike. Both were reduced in size. The capsule was slightly thickened and stripped with slight difficulty,

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† In a recent communication (*Frankfurt. Ztschr. f. Path.*, 1931, 41, 435) Elsa Adolphs describes three further cases. The first two of these cases have much in common with those already described in the literature, both as regards the changes in the spleen and also the presence of associated renal disease. In the third case, that of a child, the history was very short and, on microscopic examination, the smallest arteries and arterioles of the kidney and spleen showed acute necrotic changes of equal duration in both organs, unassociated with any older lesions. Adolphs believes that the vascular necrosis, although often occurring as a direct sequela, is to be regarded as a separate condition from the older vascular changes which are usually, though not always (Adolphs' third case), present. Further, that it is this acute necrosis, however it be produced, which is responsible for the peculiar lesion in the spleen and which plays a rôle in the fatal termination.

In and around the areas of necrosis the vessel walls are completely hyaline and structureless, and the lumina, such as they are, are occupied by thrombi which are usually of the same glassy character, rendering it difficult to differentiate thrombus from vessel wall. With the elastic stain the outlines of the vessels can be made out even in the most necrotic areas, since the elastic fibers are usually somewhat preserved.

The changes in the veins are very much less marked. In the areas of necrosis their walls are often hyaline and the lumina filled with hyaline thrombi. Apart from such regions the smaller veins show some slight thickening of the intima without any hyaline degeneration. The larger veins appear normal, but many of them contain large fresh thrombi in which the structure and the details of the cells can be observed clearly.

In the kidney the vessels show very similar changes. The larger arteries show a little proliferation of the intima and reduplication of the internal elastic lamella. In the medium-sized vessels the changes are of the same nature but more marked in degree, the lumen being greatly encroached upon by the intimal proliferation. In the smaller arteries there is a hyaline change in the intima with disappearance of the media; the elastic fibers are fragmented and stain poorly. In the adventitia of some of the vessels may be seen leucocytes of which many are necrotic and fragmented. The arterioles are hyaline and often completely occluded; some show fragments of poorly staining elastic fibers. The hyaline change in places involves the glomerular loops and many of the glomeruli show an older hyaline replacement. In others the tissue around Bowman's capsule is thickened and with elastic stain is found to contain several concentric layers of elastic fibers. Most of the tubules are collapsed and atrophic; a few are calcified. Occasionally a dilated tubule is seen containing hyaline casts or, more infrequently, blood. Around the hyaline glomeruli and the collapsed tubules is a round cell infiltration.

CASE 2. A 52 year old female, who in this case also died as a result of uremia resulting from arteriosclerotic nephritis. The kidneys were decreased in size, the capsule thickened and stripped with difficulty. The surface thus exposed was very uneven with wide gray translucent depressions and multiple small prominences. The organ was very hard and felt heavy. On section the cortex was greatly reduced and showed marked distortion of the cortical stria-

slightly thickened, as is the adventitia. The latter in one place contains a group of fragmented necrotic cells.

The smaller trabecular arteries and the larger of those that run in the follicles show more marked changes. The intima is greatly proliferated, so much so that the lumen of the vessel is frequently very much narrowed, sometimes completely obstructed. This proliferation consists of flattened fibers and elongated nuclei which lie as a rule more or less concentrically. Between the fibers there are, in many cases, numbers of leucocytes and even occasional red cells. With an elastic tissue stain the internal elastic lamella is seen to be split invariably into two or more layers. The media is thinned, apparently by compression; the adventitia is increased in thickness. In many cases in which the intimal proliferation greatly reduces but does not entirely occlude the lumen, the vessel has been finally closed by a thrombus. Most of these are fresh and their structure is easily recognized, but some are older and one is well organized.

In the still smaller arteries the walls are often completely hyaline. The endothelium may or may not be visible. Immediately outside this the tissue shows as a band of structureless clear material, staining pink with eosin, while with Van Gieson's stain it presents a yellow color. Occasionally this hyaline layer may show areas in which it is more granular and takes a mauve stain in the hematoxylin and eosin preparations: these are obviously areas of more acute necrosis. In one place there is a small mass of calcified material. The change apparently starts in the intima; the media usually cannot be made out, and whether it has been destroyed by the compression of the hyaline tissue or whether it is included in this tissue cannot be determined. The adventitia is somewhat thickened. With an elastic tissue stain it is seen that the elastic fibers within the hyaline layer are fragmented and stain very poorly. Sometimes they are altogether lacking. In the adventitia elastic fibers are proliferated. The lumen of these vessels is on the whole about normal. Many contain thrombi, most of which are fresh, though some are older and hyaline.

The arterioles have completely hyaline walls and often these walls are so thickened that the lumen is entirely lost. No separate layers can be seen with the hematoxylin and eosin stain; with elastic stain one can frequently observe a layer of elastic fibers outside the hyaline mass, apparently in the region of the adventitia. This hyaline change is often present in vessels of such small size that they may be considered as capillaries.

of the larger veins showing an organized thrombus there is a little calcification within the intima.

The necroses are similar to those described in the first case but are less frequent, smaller and on the whole fresher, with less organization around the margin. With an elastic stain the outlines of the trabeculae and blood vessels may still be seen clearly. Fat is present, both free and intracellular, at the margin of the necroses.

The malpighian corpuscles are decreased in size throughout. The sinusoids and the pulp are for the most part filled with blood, although irregular areas are seen in which the sinuses are empty.

In the kidney the appearances are those of a malignant arteriolosclerotic nephritis of great severity. The larger arteries show proliferation of the intima with occasional necroses of the media; the elastic fibers are reduplicated. The change in the arterioles is the most conspicuous; the great majority are completely hyaline with occluded lumina. Some, however, show, in addition, necrosis of the walls with hemorrhage and leucocytic infiltration into the surrounding tissues. The elastic fibers are fragmented and stain poorly. With a fat stain all these hyaline vessels are seen to contain fat in the form of fine droplets. The capillary loops of many of the glomeruli are also hyaline and occluded, and some of these glomerular tufts are adherent to the outer wall of the capsule. The tubules which correspond to such glomeruli occasionally contain red cells and leucocytes. Many of the glomeruli are completely replaced by hyaline connective tissue, and the corresponding tubules are atrophied and collapsed. In such areas there is a conspicuous round cell infiltration.

COMMENT

When an attempt is made to elucidate satisfactorily the sequence of events in the spleen and to determine the factor or factors responsible for these events, but little evidence is yet available on which to base such an inquiry. The connection between Fleckmilz and chronic renal disease, usually of arteriolosclerotic type, seems to be clear. Lubarsch³ comments on this and points out that his own four cases, together with those of Feitis¹ and Meuret,² show this association. We must, therefore, consider the changes in the spleen as a sequel or concomitant of the renal disease.

The vascular changes in the spleen and kidney have much in common. Since the deceased have, during their lives, usually shown

tions. Here and there were small bright red petechiae about 1 mm. in diameter, which suggested hemorrhages. The medulla was also reduced and the pelvis widened. Throughout the cortex the lumina even of the smallest arterioles were very clearly visible and ridgedly patent. The spleen showed but little increase in size. The capsule was slightly thickened and wrinkled. The pulp, on section, was seen to be brownish red and throughout could be seen numerous yellow or white areas, both small and large.

Microscopically, in the spleen, the changes are essentially similar to those described in the first case. However, it may be stated that the degeneration of the vessel walls is of greater degree and appears to be, in part at least, of a more acute nature than that observed in the first case. The larger trabecular arteries show a thickening of the intima and a proliferation of the elastic lamella. In the larger follicle vessels a process resembling that of a dissecting aneurysm is sometimes present. (A similar change was observed by Meuret.²) Lying beneath the endothelium and, as the elastic tissue stain shows, between the layers of reduplicated elastic fibers, are collections of cells, both red cells and leucocytes. The vessel wall in such a place is often necrotic, granular, and takes a mauve stain with hematoxylin and eosin; in other places it is pink and hyaline. In many of the follicular arteries the elastic fibers are thinned and fragmented, taking the stain poorly. Not infrequently the adventitia shows a leucocytic infiltration. In many of the vessels a hyaline fibrin thrombus is present, forming a hollow tube around the whole periphery of the lumen while the center is patent. In such a place the various coats of the wall are distinct.

The arterioles and capillaries are in the majority hyaline. Some, indeed, appear to be completely necrotic, even away from definite areas of necrosis, and take only a light pink stain. The hyaline change often occludes the lumen completely, but where this is not the case the vessel is usually plugged by a structureless thrombus. The majority of these hyaline arterioles are found to contain fine fat droplets throughout the hyaline material. The adventitia, as a rule, is thickened and contains proliferated fibers. This hyalin again stains yellow with Van Gieson's stain.

In the veins the intima is often thickened. Many of the medium and larger veins contain thrombi which are usually fresh, although sometimes hyaline, and occasionally old and well organized. In one

days' duration. It might even be suggested that the final factor leading to the thrombosis of the vessels and the production of necroses must have something in common, if it be not identical, with the factor or factors producing the death of the patient.*

The thrombosis of the veins which is present in these two cases has already been described by Lubarsch, and Feitis also found venous thrombi, albeit rarely, in his cases. These venous thrombi are even fresher than those in the arteries and in many cases must have been a terminal event. It is possible that they are secondary to the necroses to which they lie in direct relation.

CONCLUSIONS

As far as may be told from examining the changes in the spleen, the sequence of events in the cases described above would seem to be something as follows. Some toxic or other process leads to an endarteritis obliterans of the larger and medium vessels and to a hyaline change of the smaller vessels (arterioles) in the kidney and spleen. At a later date this process becomes more acute with fulminant necrosis of the vessel walls and obliteration of the medium-sized and smaller arteries by means of thrombi. In this we would agree with Meuret who came to identical conclusions in regard to the two cases which he studied. Owing to the number of vessels involved, the blood supply to areas of the spleen is shut off or reduced below that minimal amount required for the maintenance of the vitality of the tissues, so that death and necrosis occur. At the same time this exacerbation of the arterial and arteriolar disease would seem to coincide, if it is not identical, with the factors which lead finally to the death of the patient. Death, therefore, results but a short time after the onset of the necroses in the spleen which, for this reason, are mostly fresh and show only very early organization at the margin.

NOTE: My thanks are due to Professor A. Schmincke in whose laboratory this work was carried out.

* It should be pointed out, however, that the changes in the spleen may often be of very much longer standing. Thus, in two cases observed at the Johns Hopkins Hospital, Baltimore, many of the infarcts were old and well organized and the arterial thrombi were organized and recanalated. Besides these older changes there were fresh thromboses with resultant death of areas of the spleen just as in the two cases described in this paper.

symptoms of renal disease lasting for months and even years, it might not be presumptuous to suggest that in such cases the renal disease has preceded that in the spleen where the changes are often so recent. If such be the case, the splenic change may be only a later result of the unknown factor attacking the kidney; on the other hand, it may be due directly to the progressive damage to the secretory mechanism of the kidney and the resultant retention of metabolites in the blood. But whichever of these, if indeed either, is correct, there is yet nothing to show why Fleckmilz appears in some cases of chronic renal disease and not in others. One can say at least that the degree of renal change is apparently not a factor.

It seems doubtful whether the vascular changes in these spleens bear any relation to those hyaline changes which, as von Recklinghausen⁸ and more recently Herxheimer⁹ have pointed out, occur so frequently in the vessels of otherwise normal spleens. There is at the present time no clear understanding as to the significance of these hyaline arterioles which are found with increasing frequency from quite early childhood onward to old age. They have no definite relation to any particular disease or group of diseases and must perhaps be considered as a peculiarity of the spleen itself. In cases of Fleckmilz there are certain important features which differ from those to be observed in the vessels of normal spleens. The obliterative endarteritis which is so prominent in Fleckmilz is very rarely found in the "normal" arteriosclerotic spleen; moreover, the arterioles and smaller arteries in the latter condition never show the more acute necrotic and destructive changes which are present in Fleckmilz and indeed characterize the condition. The multiple thrombi which arise probably in connection with this more acute necrosis are of course a characteristic of Fleckmilz.

The fact that the hyaline arterioles stain with Van Gieson a dull yellow-red shows that the hyaline material must have been present for some time; and this for the reason that, in its early stage, such material is strongly acidophilic and takes a bright pink color, only later becoming more yellow — the color noted in these cases — and finally assuming a light canary hue. This hyaline change must, therefore, have preceded by some considerable time the thromboses and even more, the necroses of the pulp. Both of these, but especially the latter, are fresh, and this is a feature which was present also in Lubarsch's cases. Neither seems to be of more than a few

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ing a cold night. Cage mates were not affected by the weather, so that death could not be attributed to that factor alone, although, in view of the rather meager postmortem findings, it may have had a considerable part.

At autopsy the only visible changes were a mild myocardial fibrosis, a low-grade chronic enteritis, and an inconspicuous group of petechial hemorrhages on the surface of the pancreas. Only a small part of the pancreas, 1 to 2 cm., near the middle of the body of the organ was involved. The area was not diffusely congested, the organ seemed normal in consistency, fat necroses were not seen, there was no reaction in the surrounding mesentery, and regional lymph nodes were within normal limits. Hemorrhages were seen also on section through this part of the organ.

Microscopic sections of the involved area presented the following appearance. In about half of the lobules included in the section, islands of Langerhans were not seen. Acinus cells were present only in small, isolated groups, occasionally forming irregular acini but usually having no definite arrangement. These cells were small and basophilic, and nuclei were pyknotic. The parenchyma of the organ in this region was completely replaced by small, closely placed duct-like structures growing in all directions (Fig. 1). These were made up of elongate pale staining cells with large vesicular nuclei, closely resembling those of the acinus extensions of the intralobular ducts (Fig. 2). Mitoses were rarely seen. The intra- and interlobular ducts in this area were, in many instances, also lined by hyperplastic epithelium frequently growing in folds or papilliferous elevations into the lumen, with loss of cellular polarity. The interlobular fibrous tissue was definitely increased in amount and cellularity. Numerous fibrous bands passed into the lobules dividing and subdividing them. Occasional small hemorrhages associated with an infiltration of polymorphonuclear eosinophils and neutrophils were present in the areas of fibrous tissue proliferation.

The pancreatic lobules immediately adjacent to the area just described presented a transition stage between this and the nearby normal tissue (Fig. 3). Here there was a definite alteration in the appearance of the acinus and island cells. Numerous acini were distally malformed and the cells shrunken, and the islands of Langerhans appeared to be undergoing degeneration. Here also, numerous small duct-like structures similar in every way to those in the com-

TUMORS IN CAPTIVE PRIMATES *

REPORT OF TWO CASES

HERBERT L. RATCLIFFE, Sc.D.

(From the McManes Laboratory of Pathology of the University of Pennsylvania,
and the Laboratory of Comparative Pathology of the Zoölogical Society of
Philadelphia, Philadelphia, Pa.)

The infrequent occurrence of tumors in apes and monkeys, as compared to other orders of wild animals maintained in zoölogical gardens,¹ might be taken as indicating a relative insusceptibility. However, as Zuckerman² has recognized, the average span of life of the lower primates in captivity is considerably shortened by acute and chronic infections. In fact, the loss of life due to accidents of captivity reduces the average length of life to a small fraction of the "potential longevity."³ Also, as a rule, monkeys and apes are obtained as immature animals so that with the high mortality rate few live beyond the early years of maturity. Therefore, at least so far as malignant epithelial tumors are concerned, it is not surprising that the incidence is low.

In view of these facts the two cases presented here are interesting, not only because of their rarity, but also because both animals had lived in captivity considerably longer than the average for their species. Their approximate age at capture is not known.

REPORT OF FIRST CASE

The first of these cases was a female yellow baboon, *Papio cynocephalus*. The ovaries still contained active follicles when examined at autopsy, so perhaps it is permissible to put the age of this animal not beyond that comparable to the fourth decade for man. She had lived in the Philadelphia Zoölogical Garden for eighteen years and for a considerable part of this time had been kept in an outdoor cage during the entire year. No illness, loss of weight or appetite was evident prior to death, the body being found on the morning follow-

* Received for publication September 11, 1931.

REPORT OF SECOND CASE

The second case was a male Japanese Macaque, *Macacus fuscatus*. This animal had lived in the Gardens for over fifteen years but had been in poor health for two years before death. About six months before death he began to lose weight but continued to eat as usual, although he occasionally vomited after eating. Vomiting became progressively worse, so that finally he was unable to take solid food and was sacrificed.

On opening the esophagus at autopsy an obstruction was encountered at the cardia. For a space of 4 to 5 cm. the lower esophageal wall was 1 to 2 cm. thick and completely encircled by a tough, pale mass that destroyed the elasticity of the tube and caused an irregular low-lying elevation of the mucosa, so that the lumen was practically obstructed. The mass extended into the stomach as an irregular elevation of the mucosa about the cardiac orifice. Areas of erosion or ulceration were not seen. Lymph nodes adjacent to the cardia and lesser curvature were enlarged, pale gray and tough. Several nodes of the splenic group were invaded also, but there was no involvement of the liver and other viscera.

In microscopic section the submucosa and muscular coats of the esophagus and stomach were diffusely infiltrated by large groups of basophilic cells (Fig. 5). Many of these groups of cells occupied endothelial-lined spaces, probably lymphatics. In some areas there was also definite extension into the lymphatics of the gastric mucosa and the serosa. The infiltrating cells were generally of medium size, irregularly polyhedral in shape, with indefinite outlines and strongly basophilic nuclei. These cells closely resembled epithelium of the basal type. Mixed with the masses of small cells were numerous large ones with large, irregular dark staining nuclei. Occasional groups of pale cells approaching the acanthus type were also seen (Fig. 6). Mitoses, some multipolar, were numerous. These findings seem to indicate that this was a tumor of definite malignancy, apparently arising from squamous epithelium, and to justify the diagnosis of epidermoid carcinoma.

An additional finding in this animal was the presence of a diffuse low-grade hyperplasia of the bile ducts without evidence of obstruction. This was associated with cysts on the surface of the liver and may be only a part of the congenital defect represented in these structures.

pletely altered area were growing between the acini, and fibrous tissue was increased in amount and cellularity. This intermediate change was obvious enough in regions adjacent to the completely altered area and gradually faded out as the normal lobules were approached (Fig. 3). This appearance gave the impression of a spreading growth or growth-stimulus that extended from one area outwards to involve adjacent parts of the organ. This change was evident also in single lobules in which part of the tissue was relatively unchanged, the remainder being replaced by the duct-like structures (Fig. 4).

Certain features, particularly the small hemorrhagic areas with leucocytic infiltration, and the rarity of mitoses, may lead to the thought that the whole process is inflammatory. In fact this opinion has been expressed by some pathologists who have seen the sections. However, there is little to support this idea. Instead, the complete disappearance of normal pancreatic tissue in a considerable number of the lobules included in the sections; its replacement by a growth of duct-like structures; the apparent spreading character of the growth to adjacent lobules; the hyperplastic condition of the epithelium of the larger ducts, with loss of cellular polarity and the accompanying increase in fibrous tissue, all seem to indicate a definitely malignant growth and to warrant the diagnosis of adenocarcinoma. The marked infrequency of mitoses was due probably to the fact that the body was thoroughly chilled for several hours after death. The inconspicuous size of the tumor seems to be explained by considering this a chance finding of an early growth stage.

A second interesting finding in this animal was the presence of a relatively large uterine polyp, about 3 cm. in diameter, growing from the mucosa of the right tube and extending into the uterine cavity. The base of this growth was constricted, giving it a pedunculated appearance, and it seemed to be covered by an intact mucosa. In microscopic section the polyp was made up of dense cellular fibrous tissue in which numerous closely placed epithelial-lined spaces were seen. Some of these were dilated and papilliferous projections extended into them. The epithelium everywhere presented an orderly type of growth and the diagnosis of endometrial cystadenoma seems justified.

DESCRIPTION OF PLATE

PLATE 21

- FIG. 1. Case 1. A photomicrograph of a completely altered area in the pancreas. The tissue is replaced by duct-like structures. The small dark staining masses are remnants of acinus cells. The increased fibrous tissue is also shown. $\times 70$.
- FIG. 2. Case 1. Showing the cellular character of the tumor growth which consists of duct-like structures. The component cells are large and pale with vesicular nuclei. The dark staining cells are remnants of acini. $\times 390$.
- FIG. 3. Case 1. Showing a transition stage between the completely altered areas and a relatively unchanged lobule. In the upper half of the field disintegration of the parenchyma of the pancreas, and the ingrowth of the duct-like structures is seen. The section of an adjacent lobule included in the lower part of the field shows it to be relatively normal. $\times 160$.
- FIG. 4. Case 1. Showing the spread of the growth within a single lobule. The upper part of the field is the altered area, the lower relatively unchanged. $\times 160$.
- FIG. 5. Case 2. This photomicrograph is taken from a section through the gastric mucosa and shows the extensive infiltration of the submucosa by groups of epidermoid cells. $\times 70$.
- FIG. 6. Case 2. Showing a single group of the invading cells. Several of the included cells seem to approach the acanthus type. $\times 390$.

DISCUSSION

Five malignant tumors, four carcinomas and one sarcoma, have been found in monkeys dying at the Philadelphia Zoölogical Garden. These have all occurred in Old World monkeys, *Cercopithecidae*. The four animals which developed cancer had lived in captivity from eleven to eighteen years, with an average of plus fourteen years. This is more than three times the average length of life in captivity for the family.

These data are extremely scanty and those from other sources do not allow comparison. However, the present series strongly suggests that the incidence of tumors in the order primates will more nearly approach that of other groups of wild animals in captivity as more correct hygiene in zoölogical gardens becomes reflected in an increased longevity.

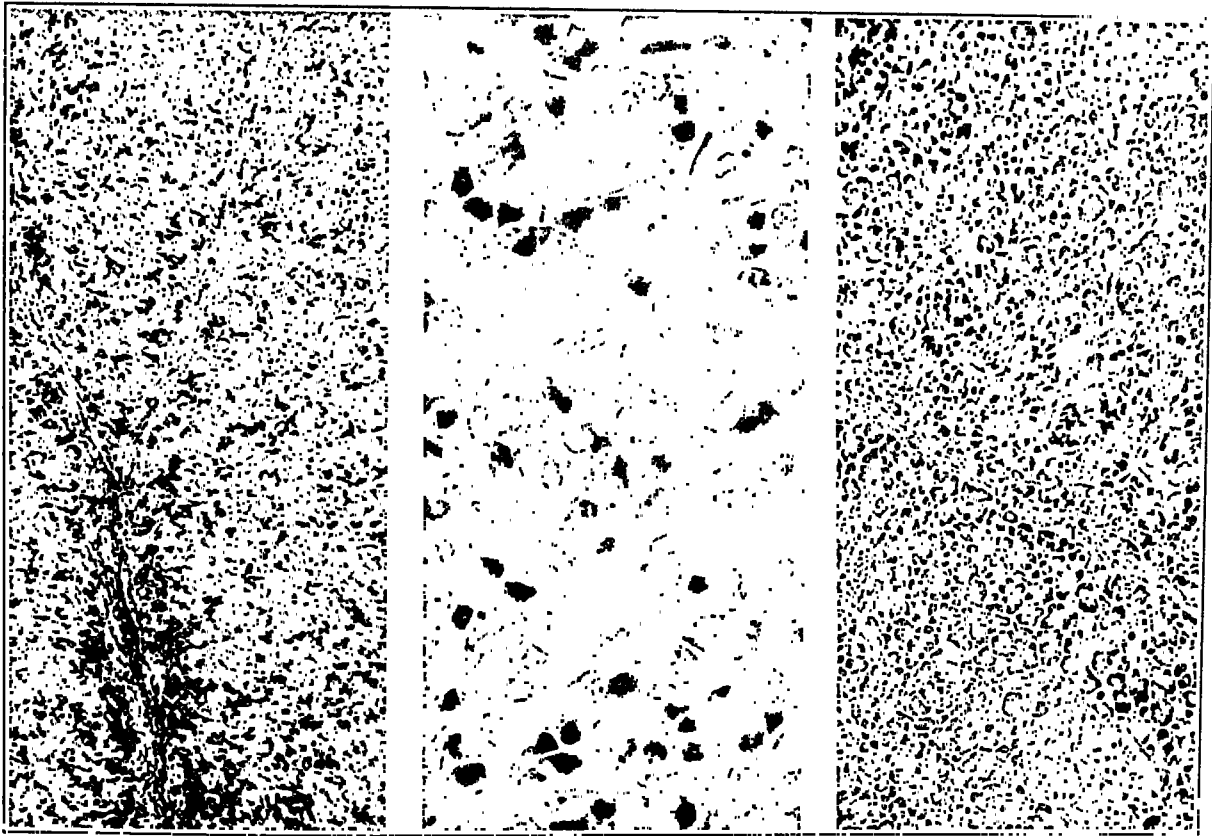
SUMMARY

Two cases of malignant epithelial tumors in monkeys are described. The first was an early adenocarcinoma of the pancreas in a yellow baboon, *Papio cynocephalus*. An endometrial cystadenoma was found in this animal also.

The second case was an epidermoid carcinoma of the esophagus in a Japanese macaque, *Macacus fuscatus*. In this animal there was also a diffuse hyperplasia of the bile ducts.

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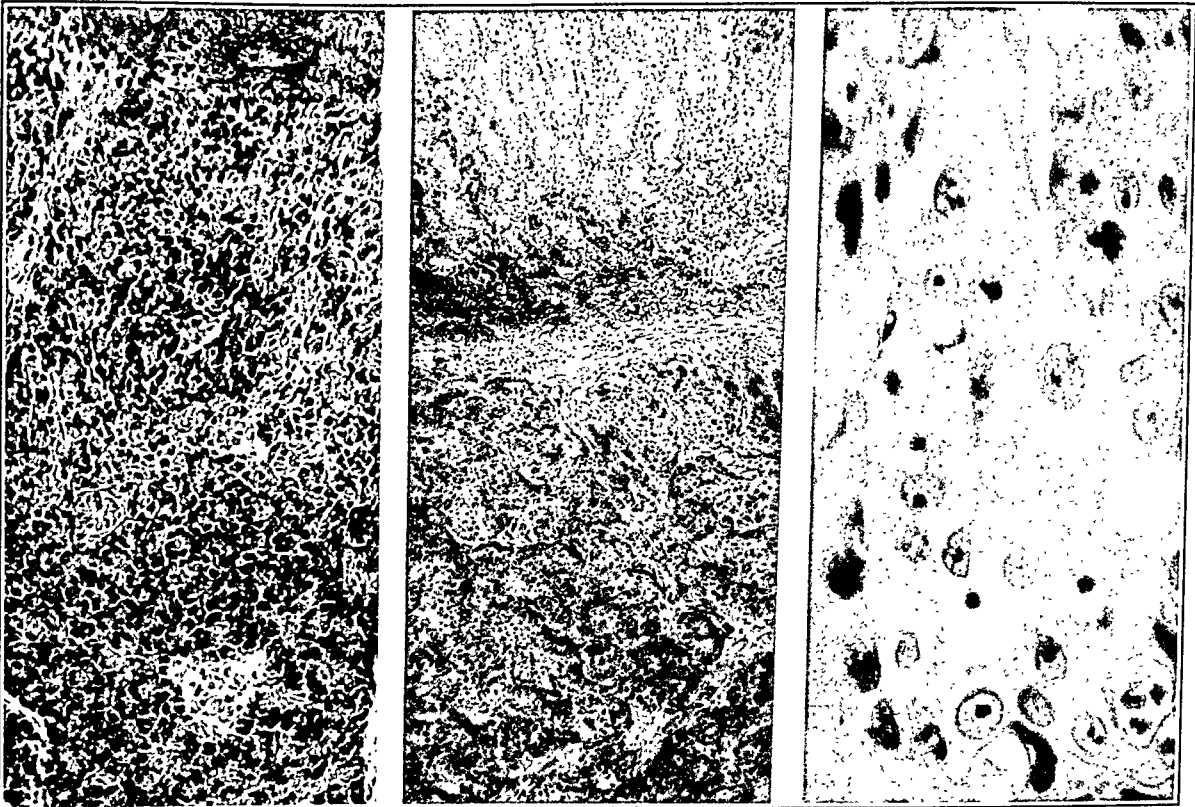
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1 micron in diameter were found. The cell body was spongy and the pole near the nucleus was filled with granules. The nucleus appeared to be separated from the cell body by a membrane. The large cells were most numerous in the interstitial tissues of the kidney, in association with areas of congenital luetic inflammation.

Löwenstein,³ working in Ribbert's laboratory in 1907, found inclusions in both parotid glands in four of thirty infants. He described without recognition the cytoplasmic, as well as the nuclear inclusions. Additional instances were reported in 1910 by Pisanò,⁴ who found inclusions in the kidneys, liver and lung, and Mouchet,⁵ who noted inclusions in the bile ducts. Pettavel⁶ in 1911 studied the thyroid of a 10 day old prematurely born infant, and described peculiar degenerative changes in the epithelial cells. Although he did not recognize the inclusions, the illustrations in his paper leave no doubt that he was dealing with the same type of inclusion bodies. This marked the first finding of the inclusions in the thyroid gland. In 1910, and again in 1914 Smith and Weidman^{7, 8} described similar findings and concluded that they were dealing with protozoa. They gave the name *Endameba mortinatalium* to the structures. Jackson⁹ in 1920 called attention to cells which she called protozoan parasites, in the ducts of salivary glands of guinea pigs. These were apparently identical with those noted in infants. Goodpasture and Talbot¹⁰ in the following year reported the finding of similar structures in the lungs, liver and kidneys of a 2 months' old infant. The salivary glands were not examined. On the basis of their study of this case, and of salivary glands of guinea pigs, they concluded that they were dealing with a new kind of abnormal cytomorphosis, to which they gave the name *cytomegalia*. They stated definitely that the structures were not protozoa and they described not only the intranuclear inclusions, but the cytoplasmic inclusions as well.

In the following year de Lange¹¹ reported the inclusions in the kidney, and Müller¹² described a similar finding in the kidneys of three infants. VonGlahn and Pappenheimer¹³ found the inclusions in the intestines, liver and lungs of a 36 year old man, for the first time in an adult. Walz¹⁴ in 1926 observed the inclusions in the pancreas, as well as in the kidneys, liver, lungs and thyroid of a newborn infant. In a discussion of Walz's paper von Albertini mentioned a similar unreported observation. The last case report was by Wagner,¹⁵ who noted the inclusions in the lungs, kidneys, liver,

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INTRANUCLEAR AND CYTOPLASMIC INCLUSIONS ("PROTOZOAN-LIKE BODIES") IN THE SALIVARY GLANDS AND OTHER ORGANS OF INFANTS *

SIDNEY FARBER, M.D., AND S. BURT WOLBACH, M.D.

(From the Department of Pathology, Harvard Medical School, and the Pathology Laboratory of the Children's Hospital, Boston, Mass.)

A routine study of the salivary glands in a fairly large series of postmortem examinations on infants has revealed a hitherto unsuspected large number showing intranuclear and cytoplasmic inclusions in the duct epithelium, often in intimate association with foci of lymphocytic infiltration. In two infants inclusion bodies were found in cells in epithelial-lined spaces in various organs of the body. These findings are included in this report.

LITERATURE

Ribbert ¹ in 1881 first noted large "protozoan-like" cells in the kidney of a luetic stillborn infant. In 1904 he published this observation, together with a description of similar structures in the parotid glands of two non-luetic infants. The large cells occurred within ducts, singly or in groups. He was preceded in publication by Jesionek and Kiolemenoglou, ² who noted the large cells in the lungs, kidneys and liver of an 8 month luetic fetus. The large cells averaged from 20 to 30 microns in diameter and were usually oval in outline with a well defined, though not sharply stained, cuticular zone having the appearance of a capsule. The nuclei were large and eccentrically placed. Each contained a "central nuclear body" surrounded by two well defined zones, an inner dark and an outer clear zone. In the clear zone deeply staining granules averaging

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TABLE I
Reported Cases with Inclusion Bodies

No.	Year	Author	Age	Location of Inclusions								Pathological diagnosis	Interpretation
				Kid- ney	Paro- tid	Lung	Liver	Pan- creas	Thy- roid	Intes- tine	Sub- lingual gland	Epidi- dymis	
1	1904	Jesionek and Kiolemenoglou	Stillborn	X	..	X	X	Congenital lues Gregarines (R. Hertwig)
2	1904	Ribbert	Stillborn	X	Congenital lues No lues No lues Amebae or sporozoa (Ehlers-Rhumbler)
3			1 yr.	..	X	
4			3 mo.	..	X	
5	1907	Löwenstein	2 mo.	..	X	Coccidia (Ludwig)
6			3 mo.	..	X	
7			10 mo.	..	X	
8			3 mo.	..	X	
9	1910	Mouchet	8 days	X	Congenital lues Sporozoa
10	1910	Pisanò	Stillborn	X	..	X	X	Congenital lues Embryonic epithelial cells
11	1910	Smith and Weidman	Stillborn	X	..	X	X	Focal nephritis Endameba mortinatalium
12	1911	Pettavel	10 days	X	Purpura Peculiar epithelial degeneration

pancreas, thyroid, epididymis and sublingual gland of a 2 weeks' premature infant, in whom no evidence of congenital lues could be found. He also found the inclusions in the parotid glands of four of a small series of infants.

An excellent review of the cases mentioned above, and a discussion of the various explanations advanced are given by VonGlahn and Pappenheimer,¹³ so that a more complete review need not be given here. The cases are summarized in Table I, which is a combination of the tables of VonGlahn and Pappenheimer,¹³ and Walz,¹⁴ with corrections and additions. It will be noted that the distribution of the inclusions in the various organs of the reported instances is as follows:

Kidneys	11 cases
Parotids.....	10
Lungs.....	8
Liver	8
Pancreas	2
Thyroid	3
Intestine	1
Sublingual gland	1
Epididymis	1

Goodpasture and Talbot¹⁰ first called attention to the similarity of these bodies to a structural variation in the intranuclear body described by Tyzzer¹⁶ in cutaneous lesions in varicella. Although the protozoa theory was kept alive in Germany until 1930 (Wagner¹⁵) a new significance was given these findings in 1921, when Lipschütz¹⁷ reported that similar structures are constantly associated with the lesions of the herpes virus in man and rabbits. Later, due to the work of Cole and Kuttner^{18,19} and to a number of intensive studies which have appeared from the laboratories of Goodpasture^{20,21} and Cowdry^{22,23} and their associates, a mass of data has accumulated to show that a definite relation does exist between inclusion bodies and certain types of filtrable virus disease (variola, vaccinia, sheep-pox, fowl-pox, molluscum contagiosum, herpes, submaxillary virus disease of guinea pigs, and so on). Goodpasture²⁰ believes that such intranuclear inclusions indicate an intranuclear localization of the infective substance in filtrable virus disease. The controversial theories and data are admirably expressed by Goodpasture²⁰ in a recent review of the subject of inclusion bodies in relation to the etiology of virus diseases.

Pearson²⁴ in a recent study called particular attention to the cytoplasmic inclusions. In a study of guinea pig salivary glands he found these inclusions to be spherical or oval in shape, and varying in size from a fraction of a micron up to 6 to 8 microns. They do not contain fat or lipoid in demonstrable amounts. Pearson had the opportunity of studying two cases from our group reported here, and found the guinea pig cytoplasmic inclusions indistinguishable from "certain cytoplasmic inclusions of rare occurrence in the human submaxillary glands." We have examined the salivary glands of a small number of normal guinea pigs and have noted on several occasions inclusions apparently identical with those found in our series of infant submaxillary glands. The guinea pig salivary gland inclusions were often accompanied by marked lymphoid infiltration.

In a discussion to a preliminary report of this study²⁵ Dr. Oskar Klotz of Toronto mentioned that inclusions similar to those described here were found by Dr. J. Thompson in the submaxillary glands in 14 per cent of a series of rats 2 months' old. These rats had been subjected to experiments on vitamin D over a short period. On the same occasion, Dr. E. V. Cowdry of St. Louis stated that Dr. G. H. Scott had found no inclusions in one hundred newborn infants and fetuses collected in St. Louis, Minneapolis and the Middle West.

DISCUSSION OF PRESENT SERIES

The submaxillary glands, and often the parotid glands, were removed in a consecutive series of autopsies on infants to determine the incidence of inclusion bodies in the salivary glands of infants, and to study the clinical and general pathological features of the cases in which these inclusions occurred. A portion of each gland removed was put immediately into sterile glycerine for further experimental work, and the remainder fixed in Regaud's fluid. Routine stains were made with hematoxylin and eosin; Giemsa and eosin-methylene blue were also employed. A study of the preparations showed the inclusions in twenty-two or 12 per cent of the 183 cases studied. In addition, two cases in which the submaxillary inclusions were noted on a previous occasion, and two others in which inclusions were found in various body organs were studied with this series, making a total of twenty-six cases available for clinical and general pathological analysis. It might be well to stress

X = present

TABLE II

Present Series with Inclusions in Submaxillary Glands

No.	Case No.	Age	Sex	Pathological diagnoses
1	A-29-245	10 mo.	Male	Miliary tuberculosis, Strep. hem. septicemia
2	A-29-246	11 mo.	Female	Idiopathic hypertrophy of heart, terminal pneumonia
3	A-30-1	8 mo.	Male	Congenital lues, Strep. hem. septicemia
4	A-30-8	12 mo.	Female	Miliary tuberculosis, tuberculous meningitis, osteomyelitis
5	A-30-39	7 mo.	Male	Pneumococcus septicemia, pneumonia, otitis media
6	A-30-52	8 mo.	Male	Strep. hem. septicemia
7	A-30-69	17 mo.	Male	Miliary tuberculosis
8	A-30-72	5 mo.	Female	Pneumonia, enteritis
9	A-30-78	17 mo.	Female	Pneumonia, otitis media
10	A-30-79	15 mo.	Female	Umbilical hernia, pneumonia
11	A-30-80	17 mo.	Female	Miliary tuberculosis, pneumonia
12	A-30-84	3 mo.	Male	Pneumonia, otitis media
13	A-30-98	11 mo.	Female	Meningococcus meningitis
14	A-30-99	7½ mo.	Female	Strep. hem. septicemia, pneumonia
15	A-30-107	3 mo.	Female	Pneumonia, otitis media
16	A-30-110	8 mo.	Male	Pneumonia, otitis media
17	A-30-111	7 mo.	Female	Meningococcus meningitis
18	A-30-117	13 mo.	Male	Miliary tuberculosis
19	A-30-181	2½ mo.	Male	Pneumonia, otitis media
20	A-30-192	6 mo.	Male	Pneumonia, otitis media
21	A-30-242	8 mo.	Male	Chronic bronchopneumonia
22	A-31-13	3½ mo.	Male	Congenital lues, pneumococcus septicemia
23	A-1009	5 mo.	Male	Keratomalacia
24	A-24-48	4½ mo.	Male	Keratomalacia

TABLE III

Present Series with Inclusions in Viscera

No.	Case No.	Age	Sex	Pathological diagnoses
1	A-30-159	20 days	Female	Hemorrhagic diathesis, inclusions in lungs, kidneys, liver, pancreas and thyroid
2	A-31-110	2 days	Male	Erythroblastosis, inclusions in kidneys, lungs, pancreas and liver

ditions in even so small a series can serve to halt, for a time at least, any speculation as to the association of the inclusion bodies with any single disease.

The fact that 80 per cent of the cases occurred in individuals under 1 year of age, that is, during a period when known diseases associated with a filtrable virus are rare, is of more than passing

the fact that in none of these cases was attention called to the submaxillary or parotid glands during life.

The clinical and pathological records of the twenty-six patients can be summarized as follows:

Age: Twenty-one instances ranged from 2 days to 1 year of age; the remaining five from 13 months to 17 months.

Sex: There were fifteen males and eleven females.

Season: The cases were scattered throughout the period of a year, with no definite seasonal preponderance.

Past History: In twenty-five of the twenty-six infants, the past history was essentially negative. There was no history of mumps or of infection in the general region of the salivary glands in any of the cases. The group includes both breast and artificially fed infants. Only one of the group had a history of contagious disease. This patient had measles, followed four months later by fatal miliary tuberculosis at 17 months' of age.

Present Illness: Vomiting and diarrhea marked the onset of illness in four cases. These were regarded as acute nutritional disturbances. In three instances the fatal illness had a sudden onset and a brief course, with death occurring in five to twenty hours. One of these had *Streptococcus hemolyticus* septicemia, one acute fulminating meningococcus meningitis, and the third died five hours after an operation for the repair of a large umbilical hernia.

There were symptoms referable to the central nervous system in several cases of meningitis due to various microorganisms, but there were no cases with unexplained manifestations of central nervous system disturbances. The remainder of the group had signs and symptoms referable to acute inflammation somewhere in the body, most often in association with the upper respiratory tract.

Clinical Course: The clinical course was variable and was usually characteristic of the particular disease. The duration of the fatal illness varied from several weeks to several hours.

Temperature: The temperature was usually high, varying in most cases from 101° to 104° F, the highest temperature occurring terminally in the instances of tuberculous meningitis.

Cause of Death: An adequate cause of death was found in every case. Five died of acute miliary tuberculosis, one having in addition a *Streptococcus hemolyticus* septicemia. Three could be grouped under the term "acute nutritional disturbance" ending with terminal infections. Bronchopneumonia and otitis media were the main features in three instances. There were two cases of pneumococcus septicemia, and two (not of the present series) of keratomalacia. Congenital lues occurred in but two patients. The other causes of death occurred singly (Tables II and III).

This summary indicates that there are no findings which would justify the grouping of these cases into a single, or even a homogeneous, clinical or pathological class. The outstanding features common to most of the group are hyperpyrexia and acute infection somewhere in the body. The occurrence of such heterogenous con-

times absent in one. Where the inclusions were numerous the ducts were often dilated, and areas of lymphoid infiltration (Fig. 1) were usually present in the immediate vicinity of the inclusion-laden ducts and acini, replacing areas of gland parenchyma. Such areas of infiltration are similar to those described by Gordon and were the most prominent accompanying pathological processes. In a few of the "negative" gland preparations areas of acute inflammation consisting of collections of polymorphonuclear leucocytes were noted, but inclusions were lacking. Where the inclusions were rare lymphocytic infiltration was usually absent, and there was no demonstrable associated pathological process. The large cells were found always in acini and ducts of the submaxillary glands, and their relationship to the lining epithelial cells appeared definite.

In the viscera the cells were always in epithelial-lined spaces — in the tubules of the kidney, the bile ducts of the liver, the acini and ducts of the pancreas, the acini of the thyroid, and the alveoli and bronchioles of the lung. They were never found free in the interstitial tissues, blood vessels, or in association with cells of other than epithelial type. This is in contrast to the observations of several of the earlier authors. No distinctive pathological process was found in these organs, the large cells often being found in otherwise normal appearing areas. In one kidney tubule there were large cells, so numerous and large in size that the lumen of the tubule appeared almost obliterated. The greatest number of large cells found in the organs of the body were in the kidney, lung and liver. Relatively few large cells were found in the pancreas and thyroid. None were noted in the intestine (VonGlahn and Pappenheimer). The epididymis was unfortunately not examined, so the observation of Wagner could not be verified.

The cells varied greatly in size, most authors mentioning a variation of from 10 to 35 microns, with an average size of 25 microns. Most often the large cells could be recognized under low powers, after some training. The shape of the cells varied from round or oval to elongated or markedly irregular outlines. Rare multinucleated cells containing inclusions were found. A sharp nuclear membrane divided the nucleus from the cytoplasm. Within the nuclear membrane was a large inclusion body which varied considerably in size, shape and staining intensity. Usually the intranuclear inclusion appeared as an ovoid or elongated, dense, homogeneous

interest. The series is naturally too small to permit any conclusions in regard to sex or seasonal incidence. The past history was essentially negative, except in one instance where measles occurred four months before death. Local factors are ruled out by the absence of a history of mumps or of any apparent lesion of the salivary glands during life. The clinical signs and symptoms were all satisfactorily explained by the clinical course and the postmortem findings. Of particular interest is the fact that there were no unexplained central nervous system disturbances. Congenital lues, with which many of the early reported cases were associated, was found but twice in this series. A listing of the cases by diseases and causes of death would show, in general, an approximate cross-section picture of the entire series from which this group was selected.

Gordon^{26, 27} in 1913, and again in 1914 reported the association of pyrexia, collapse, diarrhea and vomiting, with symptoms of meningeal irritation followed by death in from twenty-four hours to twelve days in a group of twelve children, who at autopsy showed no adequate cause for the signs and symptoms. In the salivary glands of these patients Gordon found areas of interstitial inflammation consisting mainly of lymphocytes. His descriptions and illustrations resemble very much similar areas of infiltration which were found in the vicinity of the inclusions in our material. However, Gordon states definitely that the ducts were clear, and he makes no mention of either intranuclear or cytoplasmic inclusions. He examined the salivary glands of thirty other individuals, varying from infancy to old age, and found two of that group which showed areas of lymphocytic infiltration similar to those found in his main group. These were both adults who died of peritonitis. There are no points of similarity between Gordon's group and the present series, except clinically the hyperpyrexia, and pathologically the areas of lymphocytic infiltration in the salivary glands.

DESCRIPTION OF MICROSCOPIC FINDINGS

In the series of submaxillary glands studied, the number of cells containing inclusion bodies varied from large collections scattered over many fields to single cells which were found only after a long search. Often inclusions were present in one gland only. When two blocks were taken from the same gland the inclusions were some-

SUMMARY AND CONCLUSIONS

In the submaxillary glands removed in a series of 183 postmortem examinations on infants, large cells containing intranuclear and cytoplasmic inclusion bodies ("protozoan-like bodies") were found in twenty-two cases (12 per cent). In addition, two older cases with inclusions in the parotid and submaxillary glands, and two instances in which inclusions were found in epithelial-lined spaces of the liver, lungs, kidneys, pancreas and thyroid are reported, making a total of twenty-six new cases to be added to the twenty-five already in the literature. All the patients in this series were less than 17 months of age, the majority being under 1 year. These inclusions are apparently identical with those found in the submaxillary glands of guinea pigs, and are generally similar to inclusions which are found in diseases due to filtrable viruses. Clinical and pathological studies of the series reported reveal no association with any distinctive feature or group of symptoms or disease changes. The frequency of the inclusions in our postmortem series suggests geographical factors affecting this occurrence and leads naturally to the suspicion of the existence of a disease in infants of filtrable virus etiology. However, if that be true, there are no distinctive clinical or pathological features which would permit its recognition on the wards or in the pathology laboratory. The clinical and pathological findings in the "positive" instances resemble, in general, the findings in the entire group studied.

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acidophilic body. Often the body stained more deeply in the central portion and shaded off slightly to a paler portion at the periphery. The outline of the body was usually not sharp. In some instances delicate honeycombing to coarse vacuolization could be observed within the inclusion body. No finer structures could be recognized. Surrounding this body, and between it and the nuclear membrane, there was usually a clear zone which varied in size with the outline of the nuclear inclusion body. Occasionally the clear zone was entirely obliterated by the encroachment of a vacuolated, swollen, nuclear inclusion body. In the clear zone there were usually one or two, sometimes three or four small, round to oval or spherical, dense, basophilic, granular masses, which in some cases had apparently fused to form irregularly shaped, densely staining clumps. In rare instances, in the cells of comparatively small size, no definite nuclear body was found. In the clear zone of such nuclei twenty to thirty small, densely staining masses of chromatin material were scattered, sometimes distributed in almost concentric arrangement, and at other times gathered in groups adjacent to the nuclear membrane. This we regard as an early stage in the formation of the inclusions. One perplexing feature in our study of the inclusion bodies was the failure to find small forms which could with confidence be interpreted as stages in formation. If the inclusions were present at all they were strikingly alike and within a constant narrow range of size and detail.

The cytoplasm of the cells with inclusions was basophilic in staining reaction, and usually contained a few to large numbers of dense, basophilic, oval to spherical granules which varied greatly in size. Often these cytoplasmic inclusions appeared almost round in shape, and were arranged in curved rows, conforming to the shape of the cytoplasm. The cytoplasmic inclusions were present in almost all of the large cells in the submaxillary glands, and in most of the large cells in the organs of the body. Often, when they were apparently lacking, better stained sections would bring the cytoplasmic inclusions out more clearly. The cytoplasmic inclusions do not represent mucus droplets, as might be at first suspected. Microchemical studies were carried out by Pearson,²⁴ who found that specific tests for mucin yielded rather inconclusive results. Furthermore, we have repeatedly observed cytoplasmic inclusions in epithelial cells lining structures where mucus-secreting cells normally do not occur.

DESCRIPTION OF PLATES

PLATE 22

FIG. 1. Photomicrograph of submaxillary gland. Note areas of lymphoid infiltration. Hematoxylin and eosin. Low power.

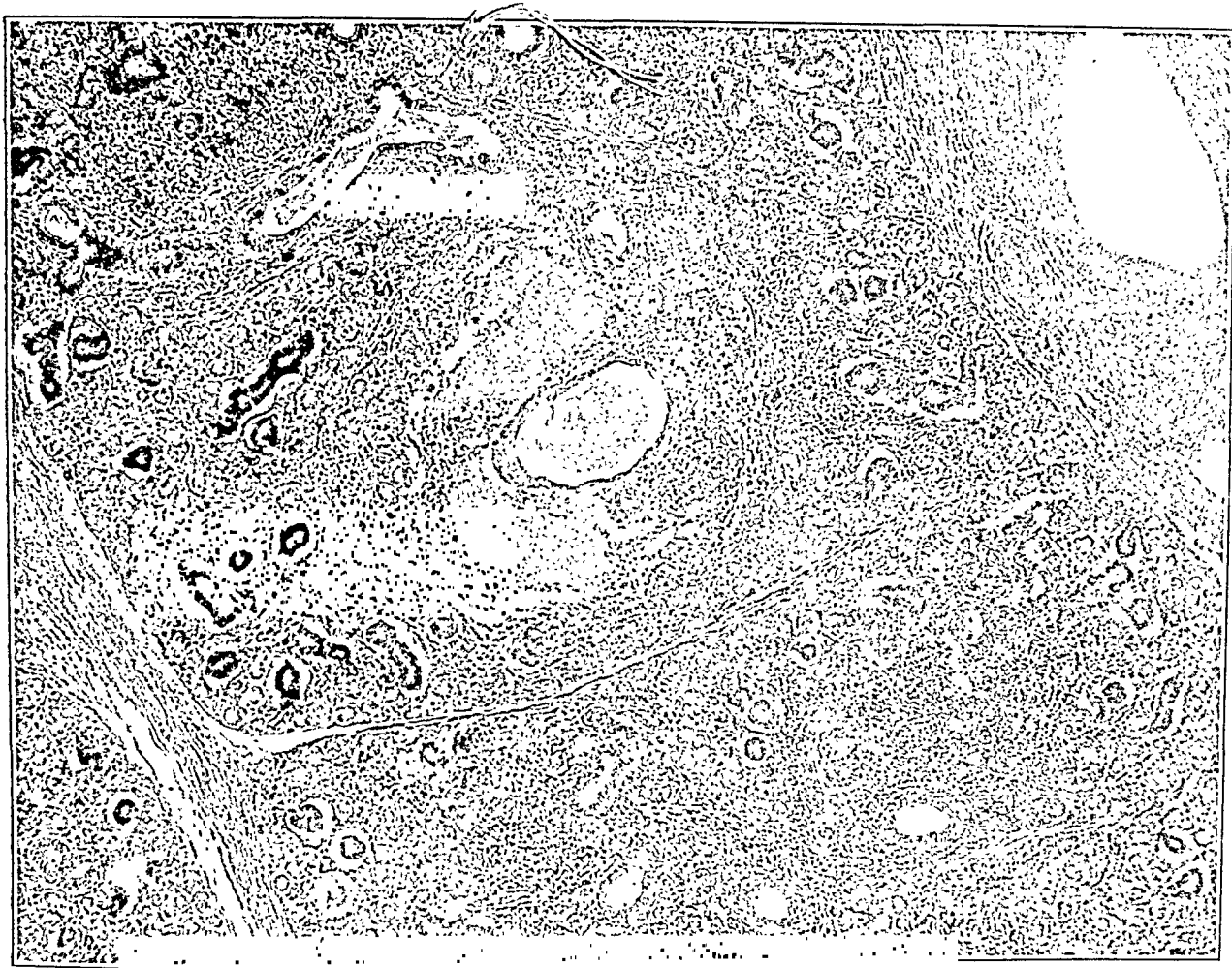
FIG. 2. Photomicrograph of submaxillary gland showing large cells with inclusions lining the duct. Hematoxylin and eosin. $\times 550$.

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PLATE 23

FIG. 3. Photomicrograph of kidney showing large inclusion-laden cells lining the kidney tubule. Note the swollen cytoplasm and vacuolated appearance of some of the cells. Hematoxylin and eosin. $\times 750$.

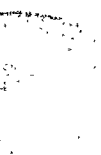
FIG. 4. Photomicrograph of submaxillary gland showing a large cell in the duct lining. Note intranuclear inclusion, pale area at periphery, clear zone, small masses in clear zone, nuclear membrane and large inclusion bodies in cytoplasm. Hematoxylin and eosin. $\times 2300$.

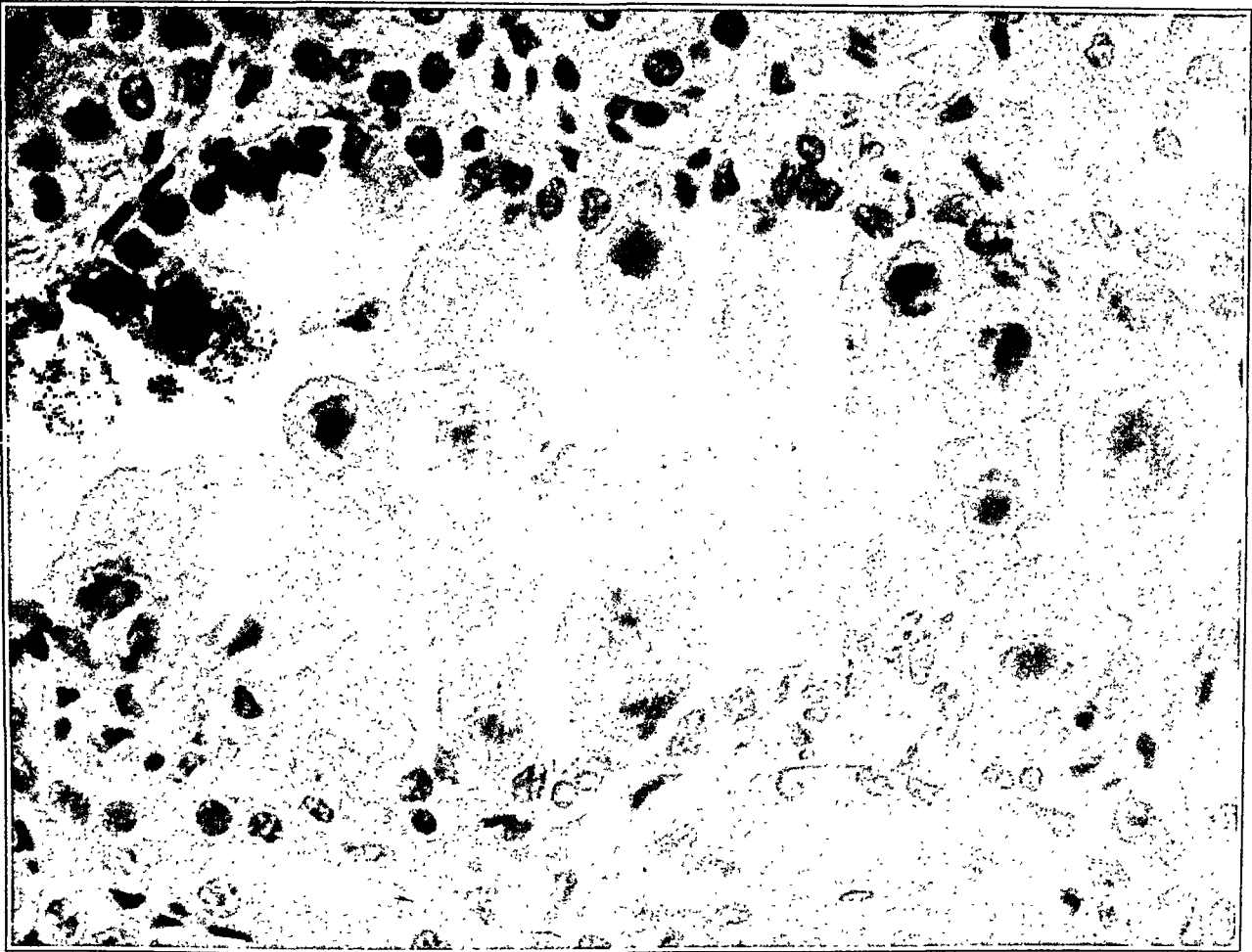


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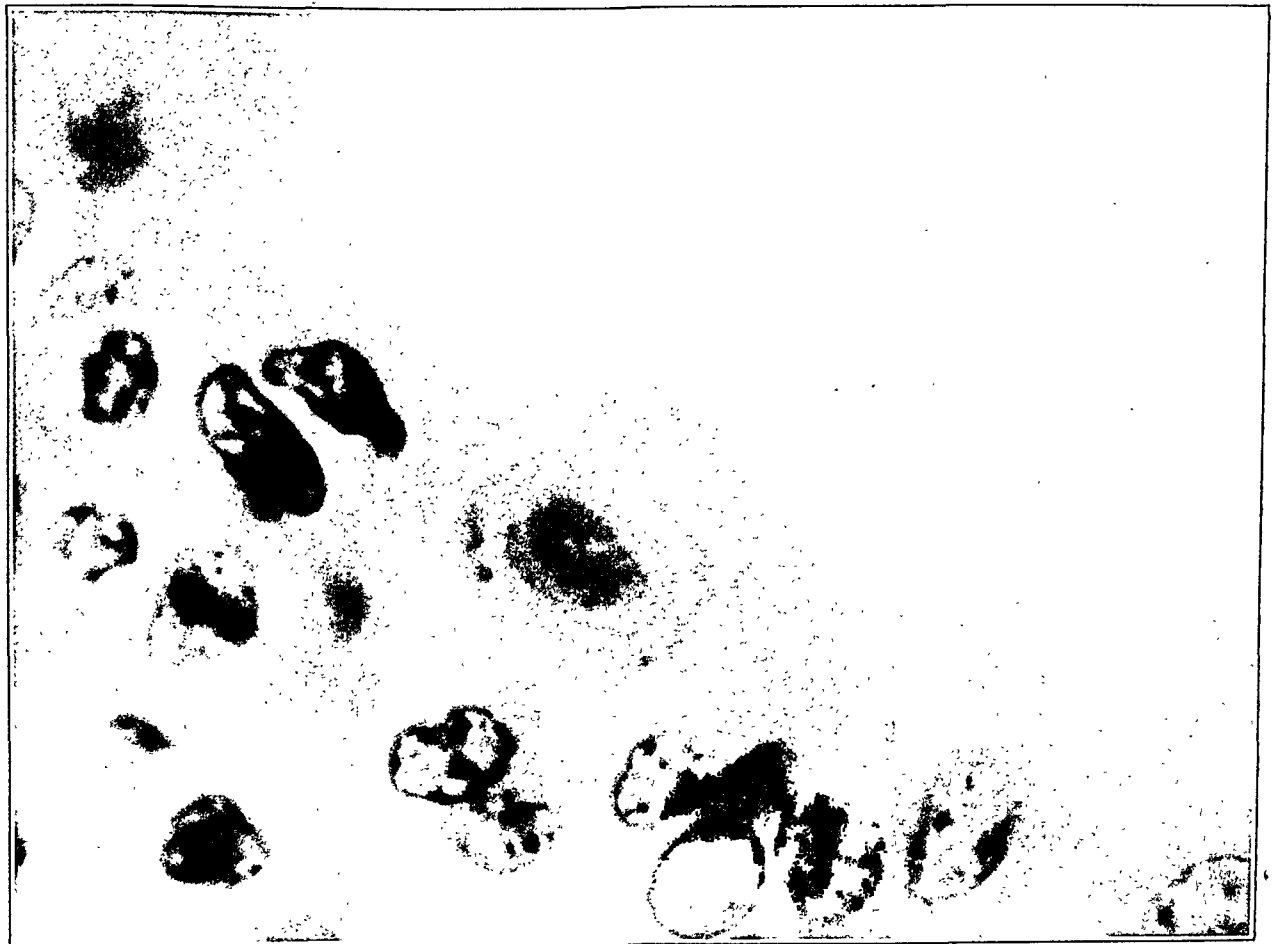


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cytotropic filterable viruses should be known to reproduce themselves locally in association with the presence of cellular inclusions.⁴ This evidence was lacking, inasmuch as the inclusions had been found only in the liver cells, and there seemed to be no evidence that virus was regenerated locally in the liver in association with them.

This essential condition seems now to have been satisfied through the extraordinary discovery by Theiler⁵ that the virus of yellow fever appears to be infectious for the brain of mice; and his experiments indicate it may be successfully cultivated in series in this tissue, with the induction of a fatal encephalitis. Theiler's observations further show that the mouse encephalitic virus is essentially restricted in its distribution in fatal infections to nervous tissue and adrenal gland, which has a considerable nervous component in its medulla. The virus, according to his observations, also passes centrifugally from the spinal cord along peripheral nerves. These facts definitely relate the mouse strain of virus to other neurocytotropic viruses, namely those of rabies, poliomyelitis, enzoötic encephalomyelitis and herpes simplex.⁶

Furthermore, the recent investigations of Sellards⁷ contribute the important information that the mouse virus passed serially through brains of mice becomes modified in its action upon monkeys (*M. rhesus*), in that it will then induce a fatal encephalitis in these animals when inoculated intracerebrally, without causing the usual symptoms and hepatic changes of the original yellow fever virus.

Theiler was rightly cautious in his attitude toward the question of the identity of the mouse encephalitic virus and that of yellow fever, notwithstanding the fact that inclusions quite similar to those found in the liver in the natural and experimental disease were to be observed in the central nervous system of the infected mice, and, what is more significant, that yellow fever immune serum from both monkey and man showed protective power for mice inoculated with the encephalitic virus.

Evidence of the identity of the mouse virus and that of yellow fever is further contributed by the investigations of Sellards, who showed that monkeys can be immunized against typical yellow fever virus by intraperitoneal injections of mouse virus, and that monkeys immunized to typical yellow fever virus manifest a well marked, though not entirely complete protection against intracerebral injection of virulent mouse virus. Sellards concludes that the results

YELLOW FEVER ENCEPHALITIS OF THE MONKEY (MACACUS RHESUS) *

ERNEST W. GOODPASTURE, M.D.

(From the Department of Pathology, Vanderbilt University Medical School,
Nashville, Tenn.)

Since the discovery by the American Commission at Havana in 1900 that the etiological agent of yellow fever is filterable, this infection has generally been grouped tentatively with the virus diseases. In later years this classification has seemed more insecure because it has become apparent that certain bacteria, protozoa and spirochetes may pass through similar filters. The finding of leptospiras by Noguchi in a group of cases clinically diagnosed yellow fever made it seem for a while still less likely that the causative agent belongs to the virus group.

Recently, however, a mass of evidence has been gathered which seems to place the active agent of yellow fever not only among the filterable viruses, but also with the cytotropic group of these infectious agencies. The recent rapid accumulation of this evidence resulted directly from the discovery by Stokes, Bauer and Hudson ¹ that yellow fever may be transmitted with regularity to the Indian monkey, *Macacus rhesus*. With this animal available for the experimental study of the disease many important facts have come to light.

Bearing upon the viral etiology of yellow fever was the absence of leptospiral infection in the West African cases, the reconfirmation of the filterability of the active agent in the blood stream, and the discovery by Torres ² that intranuclear inclusions are to be found in the injured cells of the liver of experimentally infected monkeys. Shortly afterward similar inclusions were described by Cowdry and Kitchen ³ in liver cells of human cases of West African yellow fever.

In commenting upon these important discoveries I recently expressed doubt that the agent of yellow fever should be classified as a cytotropic virus, even though these two basic facts of filterability and specific cellular inclusions were available, for the reason that

* Received for publication December 19, 1931.

inclusions depicted by Torres and by Cowdry and Kitchen. The impressions gained from a study of these preparations served as a guide to the study of the encephalitic lesions.

Sections of brain from the two monkeys in Group I, which received typical yellow fever virus intracerebrally, show no evidence of encephalitis or of intranuclear inclusions. The livers of both animals contain the typical necrosis of yellow fever infection. As pointed out by Sellards, the typical yellow fever virus, even though introduced directly into the substance of the brain, brings about the usual appearance of yellow fever uncomplicated by a viral encephalitis.

Sections of the brains of mice dead of encephalitis show, as first described by Theiler, a perivascular mononuclear cellular exudate particularly marked in my preparations in the basal ganglia. In the brain of a young mouse, dead six days after inoculation, abundant nuclear inclusions were observed both in the ganglion cells of the cerebrum and those of the basal ganglia. It was noted that extensive necrosis of ganglion cells accompanied the presence of inclusions, and this without any evidence of cellular exudate. It seems evident from a study, both of the encephalitis of mice and of monkeys, that, as in other neurocytotropic virus lesions, the first change is in the ganglion cells, and inflammatory exudate is secondary, apparently to cellular necrosis. In an adult mouse brain perivascular infiltration and focal inflammatory exudate are prominent, but only a few inclusions were observed. This single observation suggests, on cytological grounds, that the brains of young mice are more susceptible to the virus.

The intranuclear inclusions observed in the brains of mice correspond in appearance in every way to the now well known descriptions of Torres and of Cowdry and Kitchen. Following is a description of lesions found in the brain of two mice, the first a baby mouse dead on the sixth day after inoculation, the second an adult mouse.

BABY MOUSE — 6TH DAY

Cerebral Cortex and Basal Ganglia: (Stained with methylene blue and eosin.) Changes in ganglion cells throughout the sections are to be seen in great abundance. They are more numerous in the basal ganglia, but are also diffusely scattered through the cerebral cortex.

of these cross-immunity tests are entirely consistent with the interpretation that the virus in mice is that of yellow fever, and there is no indication that it is contaminated by any secondary virus. He states however that the amount of data available at present is not overwhelming and there is no urgent need for drawing any altogether final conclusions. In regard to hepatic lesions in monkeys infected intracerebrally with mouse virus he states: "Of five normal monkeys injected into the brain with mouse virus, none showed lesions of the liver comparable to the changes which occur in man or in monkeys dying of typical yellow fever. In one of these monkeys, a moderate amount of necrosis of the liver was found, and in another, the liver was normal. In the remaining three monkeys the liver showed moderate degenerative changes consistent with the earlier changes seen in yellow fever but by no means diagnostic and quite unlike the extensive necrosis seen in monkeys dying in the usual manner."

For a more detailed study of the cytology and histology of mouse virus encephalitis in mice and monkeys, Dr. Sellards kindly sent to me stained sections and blocks of tissue from mice and monkeys infected with this virus, and in addition, for comparison, sections of human livers from West African cases of yellow fever, and of livers from monkeys experimentally infected with yellow fever virus. This report is based entirely upon a study of the material which Dr. Sellards made available to me for this purpose.

In addition to the sections of human and monkey livers infected with yellow fever and sections and tissue from the brains of encephalitic mice, there was material from the following groups of experiments upon monkeys.

GROUP I: Included tissue from two monkeys which had received intracerebral injections of the typical monkey strain of yellow fever virus.

GROUP II: Included tissue from five normal monkeys inoculated intracerebrally with encephalitic virus from mouse brains.

GROUP III: Included tissue from four normal monkeys inoculated intracerebrally in series with virus originating from a monkey dead of mouse virus encephalitis.

In sections from the livers of one human and two monkeys, stained with methylene blue and eosin, intranuclear inclusions were found which correspond in all respects to the description of yellow fever

neuronic degeneration and necrosis. There is no exudate in the meninges. The cellular degeneration is diffuse, extensive and bilateral.

Cerebellum and Pons: Similar cellular changes are abundant in the pons. No definite changes are seen in the cerebellum. Purkinje cells contain much granular eosinophilic coagulum in the nucleus, but this appears to be normal.

ADULT MOUSE BRAIN

Section Through Cerebral Cortex, Basal Ganglia and Ammon's Horn: One can easily recognize under low magnification that there is a diffuse encephalitis throughout the midcerebrum. This is indicated by an abundant perivascular cellular infiltration, very marked in the basal ganglia, and inconspicuous in the cortex. There is moderate round-cell infiltration in the meninges at the base of the brain. The inflammatory lesions are bilateral in distribution.

Blood Vessels: The blood vessels, including capillaries, are congested and occasional punctate hemorrhages are seen in the basal ganglia.

There is an abundant perivascular cellular infiltration about the larger veins. The cells are all mononuclears, some of them are lymphocytes, but most are wandering cells. No polymorphonuclear leucocytes are seen. In addition to the perivascular exudate there is also a diffuse invasion of parenchyma by mononuclear wandering cells. This is most abundant about the mantled veins and capillaries. An occasional polymorphonuclear leucocyte is seen in the parenchymal exudate. Now and then a mitotic figure is found in a neuroglial cell.

Cellular Changes: Despite the abundant perivascular and diffuse cellular exudation, neuronic alterations are difficult to find. There are occasional necrotic cells, but very careful search is necessary to discover a nuclear inclusion. They are almost negligible in number in the cerebral cortex and midbrain where perivascular infiltration is most marked, but are fairly numerous and typical in Ammon's horn, where there is no inflammatory exudation.

In the cortical cells the changes are largely nuclear, though occasionally shrunken, acidophilic necrotic cells are found. In the basal ganglia necrosis of cells is conspicuously in evidence. The necrotic cells occur in irregular groups.

The common and conspicuous nuclear change consists in the presence of masses of amorphous, finely granular, acidophilic material within the nucleus, associated with granules of basophilic material irregular in size. Sometimes, though less frequently, the acidophilic mass is single, occupies the center of the nucleus and is separated from the nuclear membrane by a clear zone.

More frequently, however, the acidophilic material is found in several masses, either almost filling the nucleus, or separated from the nuclear membrane by a clear zone. It is quite characteristic of these acidophilic inclusions that they incorporate amorphous granules of basophilic material. At times there is a large granule of basophilically-stained material which suggests a nucleolus. The central eosinophilic mass is not usually separated so clearly from the nuclear membrane by a clear zone, neither is the aggregation of chromatic particles upon the nuclear membrane so characteristic as in herpes. Quite commonly the rarefied zone about the inclusions is not distinctly clear. The acidophilic material seems finely granular in composition and only loosely adherent.

Occasionally one finds the entire nuclear content apparently coagulated into a coarsely granular clump in the center, separated from the nuclear membrane by a clear zone. These nuclear clumps stain basophilically and do not seem to be identical with the commoner acidophilic inclusion. I have observed similar clumping of basophilic nuclear material in sections of brains from apparently normal fowls. Only ganglion cells contain inclusions. No change is observed in neuroglia, ependyma, choroid plexus or endothelium.

Blood Vessels: These are generally congested, and punctate hemorrhages are found both in the cortex and basal ganglia, but more frequently in the latter.

Exudate: There is no diffuse cellular exudate. About some of the small distended veins of the basal ganglia there are collected a few mononuclear cells difficult to classify, and there is an occasional small group of mononuclear cells in a focus related to necrotic ganglion cells. In a superficial inspection of the section one would hardly detect any cellular exudate at all, notwithstanding the extensive

tensive degeneration, necrosis and inflammatory exudate. In the nuclei of relatively intact ganglion cells showing chromatolysis occasional distinct acidophilic inclusions were observed. These sometimes correspond in appearance to the yellow fever inclusion in general. Other nuclei, perhaps more commonly, contain in addition to the nucleolus one or more compact, spherical or oblong, pink-staining, homogeneous masses somewhat larger than nucleoli, situated in the center of the nucleus and separated from the nuclear membrane by a clear zone. Upon the nuclear membrane lie particles of basophilic material. These inclusions are not typical of yellow fever.

Many ganglion cells are shrunken and necrotic, and apparently they may reach the stage of necrosis without exhibiting the change characterized by inclusions. Early in the degenerative stage mononuclear phagocytic cells appear about the periphery of the cell, and soon entirely replace it. Occasionally a mitotic figure is seen which seems to be in a neuroglial cell. Of especial interest is the observation that not infrequently an acidophilic inclusion is to be found within the nucleus of one or more of the mononuclear inflammatory cells which are phagocytizing a ganglion cell. These structures are round or elongated, and are about the size of a nucleolus, but distinct from it. They appear more dense, concrete, and refractive than the acidophilic inclusions generally seen in the ganglion cells, but are no more so than others that are occasionally found.

Polymorphonuclear leucocytes are rarely found in this section. There is a diffuse infiltration of the affected gray matter by mononuclear wandering cells with pale irregular nuclei, and a slight perivascular accumulation of similar mononuclears and lymphocytes. Veins are conspicuously distended.

Right Hemisphere and Brain Stem: One-half the brain, including the pons and medulla, was available in formalin, to study the distribution of lesions on the side opposite that receiving the inoculation.

Transverse sections including the entire half brain were cut through the frontal, parietal and occipital lobes, and through the cerebellopontine portion and through Ammon's horn. A diffuse encephalitis with lesions similar to those described, though varying in extent, was found in the gray matter from the frontal region through the pons. In some areas, such as Ammon's horn, the inflammatory exudate consists almost entirely of polymorphonuclear leucocytes.

ENCEPHALITIS OF MONKEYS

No distinct differences could be noted between the severity, extent or general characteristics of the lesions in the brains of monkeys, whether they received directly the virus from infected mouse brains, or serial inoculations of brain from other monkeys infected with the mouse virus. Each of the brains of Groups II and III shows an extensive, severe, bilateral acute encephalitis which affects especially the gray matter, and in general seems most intense in the basal ganglia and pons. Altogether there were nine monkeys in these two groups. In addition to sections of the brain there was also tissue from the spinal cord from five of the nine monkeys. In four of these there is a severe, destructive, acute myelitis affecting the entire gray matter of the cord, but particularly involving the motor ganglion cells of the anterior horns. In one spinal cord no inflammatory changes were observed. The sections, however, were taken from one level only.

An illustrative protocol of the encephalomyelitis in monkeys follows:

RH-282. MOUSE BRAIN TO MONKEY BRAIN

Cerebral Cortex: In the plane of inoculation there is an extensive degeneration and necrosis of ganglion cells. The nuclei of many of these cells contain rather coarse acidophilic clumps or inclusions. It is difficult or impossible to recognize in these inclusions anything characteristic of yellow fever. Certainly one would hesitate to make a tentative diagnosis of yellow fever encephalitis on that basis.

There is a diffuse, though moderate polynuclear leucocytic infiltration of the cortical tissue, and to a less extent a mononuclear wandering cell exudate. About many blood vessels there is a thin perivascular mantle of mononuclear cells. The vessels generally are greatly dilated and distended. Petechial hemorrhages are numerous. Edema is evident. There is no meningitis.

Cerebellum and Pons: The cells of the cerebellum show no recognizable changes. The pyramidal cells of the normal monkey's cerebellum contain relatively conspicuous eosin-staining clumps and granules about the nucleolus. There is no inflammatory exudate in the cerebellar cortex.

The gray matter of the pons beneath the cerebellum shows, particularly within and about groups of large ganglion cells, an ex-

myelitis in man and monkey, rather than that of rabies and Borna disease. Like herpes and poliomyelitis the mouse virus causes a very acute fulminating disease, acutely destructive of ganglion cells. Unlike poliomyelitis, however, the injury is not so restricted in its distribution and affects both sensory and motor cells. In its distribution and its action upon both sensory and motor neurons it is more like the herpetic encephalitis, as seen in fulminating infections of rabbits. Unlike herpes, however, the mouse virus does not seem to affect the meninges, and its lesions are not so focal.

NUCLEAR INCLUSIONS IN MOUSE VIRUS ENCEPHALITIS OF MONKEYS

Of especial interest in this study is the cytology of the neurons affected by the virus, with particular reference to the occurrence of intranuclear inclusions in the brains of monkeys. The observation by Theiler that intranuclear inclusions similar to those described in yellow fever occur in the brains of infected mice, and the presence of such inclusions in the mouse brains sent to me by Dr. Sellards, led to the expectancy that the mouse virus encephalitis of monkeys would be easily distinguishable from other forms of acute encephalitis by the presence of specific inclusions in ganglion cells. This, however, did not prove to be the case. It is true that intranuclear inclusions have been found in my preparations, but they are usually detected with difficulty, are few in number, and are often distinctly different morphologically from the typical intranuclear inclusions of yellow fever livers and mouse brains infected with the yellow fever virus. All of the inclusions observed were intranuclear and they were found in only five of nine cases of encephalitis in monkeys. My search was not exhaustive, however, and an investigation of more slides from different blocks of tissue possibly would have revealed a higher incidence. The inclusions found impress one as being of viral origin and occasionally they appear typical of yellow fever. The variations from type were found particularly in large multipolar ganglion cells, in one case the motor ganglion cells of the anterior horns of the spinal cord. In such cells there is chromatolysis and an eosinophilic staining of the cytoplasm. The nucleus is perhaps slightly enlarged. The nucleolus is partially or completely preserved, staining with methylene blue. About the nuclear mem-

Spinal Cord: There is ganglionic necrosis in both ventral horns. Several of these cells are being phagocyted by mononuclear phagocytes. There is a diffuse inflammatory exudate consisting of both polynuclear and mononuclear leucocytes. Edema and petechial hemorrhages are found.

Comment: An examination of these nine monkey brains shows that the strain of virus derived by inoculating mice intracerebrally with yellow fever virus and passed serially through these animals is an exceedingly destructive infectious agent for the central nervous system of normal monkeys, whether the virus is introduced directly from the mouse or passed serially through the brains of monkeys. One is led to judge that the virus rapidly traverses the central nervous system from the site of inoculation, causing an intense encephalomyelitis. The meninges do not seem to be involved in the inflammatory process.

The infectious agent attacks primarily, if not exclusively, the neurons (both sensory and motor), resulting in rapid degeneration and necrosis of these cells before inflammatory exudate appears. Associated with the injury to ganglion cells there is congestion of capillaries and veins, an inflammatory edema, and focal hemorrhages.

In what seem to be unusually severe acute lesions polymorphonuclear leucocytes make their appearance early and in considerable numbers before mononuclear phagocytes are to be found. Not only may there be a diffuse distribution of polynuclears in the inflamed area, but not infrequently they localize about dead ganglion cells. More commonly, however, there is an admixture of large mononuclears or the cellular exudate is composed of them entirely. Ordinarily phagocytosis of dead ganglion cells is accomplished by the mononuclears entirely. It is in these inflammatory cells collected about or replacing dead ganglion cells that one occasionally sees an acidophilic intranuclear inclusion, the significance of which is not apparent. If the lesion is of sufficient duration the veins become mantled by an accumulation of mononuclear cells, for the most part lymphocytes.

It seems evident in these preparations that ganglionic injury and necrosis is the first manifestation of the destructive effect of the virus in the nervous system, and inflammatory reaction, including cellular infiltration, is secondary.

In comparison with other neurocytotropic virus lesions, the mouse virus encephalitis resembles that of herpes in the rabbit and polio-

viruses induced by experimental procedures, such as the modification of smallpox virus by passage through the calf. Such changes, however, represent apparently only variations in virulence, not of cytotropism. The virus of herpes simplex affords an instance of a profound divergence in cytotropic affinity of a virus in different species, as manifested by strains which possess a predilection for the skin in human beings and nervous tissue in the rabbit. The herpes virus, passed serially through the brains of rabbits, according to the experiments of Teissier, Gastinel and Reilly,⁸ tends to lose its infectiousness for the human skin, but there is no indication that herpes virus becomes thereby more neurotropic in the human.

There is no reasonable doubt that Theiler's mouse virus is a neurocytotropic virus. It corresponds in its pathological activity and tissue affinity to the viruses of rabies, poliomyelitis, Borna disease and herpes. It is rather unexpected therefore that the mouse virus inoculated into the brains of the monkey (*M. rhesus*) does not induce more characteristically the cellular changes found in the brains of mice infected with the virus of yellow fever. However, there is a variation in the observed incidence of the yellow fever inclusions in the livers of human beings and of monkeys. The most distinctive difference between the effect of the mouse virus in the brains of mice and in the brains of monkeys is in the morphology of the intranuclear inclusions. Although one occasionally finds inclusions in encephalitic monkey brains which may be interpreted to be similar to those of mouse encephalitis, it is more common to find intranuclear inclusions, apparently of viral origin, which differ from them. An intranuclear inclusion in the monkey encephalitis atypical of yellow fever is a more compact, homogeneous, and discrete acidophilic mass somewhat suggestive of that found in Borna disease, though usually larger.

It should be borne in mind, however, that there is considerable morphological variation in most inclusions, though similar variations occur in each tissue regardless of the species of the host. The yellow fever inclusions are especially difficult to diagnose with certainty in their finely granular form as observed in fixed tissue, because they resemble so closely the granular precipitate from nucleoplasm which may be observed in many normal nuclei. They may be recognized with certainty, as is true also with the herpetic inclusions, only in their well developed forms, and when they occur in numbers.

brane are aggregated amorphous basophilic particles. The remainder of the nucleus appears empty except for one or more spherical or oblong, homogeneous, hyaline, pink-staining masses, usually larger than the nucleolus. Sometimes there are in addition to these structures smaller aggregations of minute pink particles resembling the material which constitutes the typical yellow fever inclusions. This description is based upon sections fixed in Zenker's solution and stained with methylene blue and eosin. In smaller ganglion cells the central area of the nucleus is sometimes found to be filled with pink-staining granular material, with chromatin particles collected upon the nuclear membrane. In these cells no nucleolus could be detected. Usually necrotic cells show no evidence of inclusions.

In this investigation only the staining reaction of inclusions with eosin, and their morphology, have been considered, partially because of the limited possibilities of the material at my disposal. In an exhaustive and detailed investigation of the yellow fever inclusions of human and monkey livers Cowdry and Kitchen were unable to detect any microchemical differential characteristics of these structures, and they finally relied largely, as did Torres, upon morphological configuration for evidences of specificity.

DISCUSSION

The discovery by Theiler that the encephalitic brains of mice inoculated with the virus of yellow fever contain intranuclear bodies similar in every way to the inclusions previously described by Torres, and by Cowdry and Kitchen in the livers of monkeys and human beings dead of this disease, is very strong evidence that this cellular change is a characteristic effect of yellow fever virus upon cells. Especially significant is the fact that similar changes are brought about in two tissues so different as those of the liver and the brain. This cytological characteristic of the lesion, together with the immunological data supplied by the experiments of Theiler and of Sellards, makes it seem very probable that the virus of mouse encephalitis induced by the inoculation of typical yellow fever virus is in reality a modified form of the active agent of yellow fever.

It seems to be a unique phenomenon that a virus can become so distinctly and rigidly changed in its tropism or cellular relationship, although experience affords several instances of the variability of

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DESCRIPTION OF PLATES

Magnification 2300, except Fig. 3, which is 60 diameters.

PLATE 24

- FIG. 1. Ganglion cells from baby mouse's brain. Intranuclear granular acidophilic inclusions interspersed with basophilic granules. The larger ones may be nucleoli. These inclusions are like those of yellow fever livers.
- FIG. 2. Ganglion cell from baby mouse's brain. Intranuclear mass largely composed of basophilic granular material. Atypical of yellow fever.
- FIG. 3. Monkey encephalitis, to show perivascular and diffuse cellular infiltration.
- FIG. 4. Ganglion cell from monkey encephalitis showing nucleolus (dark sphere) and acidophilic granular material rather loosely arranged, resembling yellow fever inclusions.

The difference in structure of the typical intranuclear inclusions of the encephalitis of monkeys inoculated with mouse virus may represent, therefore, another variation in the activity of this virus not commonly seen in viral diseases.

In consideration of the protection experiments of Theiler and of Sellards, and of the fact that intranuclear inclusions quite like those of yellow fever are demonstrable in the cells of mouse brains inoculated with yellow fever virus, and finally that a fatal viral encephalitis may be induced in monkeys by the intracerebral injection of virulent mouse brains, characterized by the presence of intranuclear inclusions (some of which may resemble those of yellow fever) one feels that the evidence, both immunological and cytological, favors the view that the mouse virus represents a modified strain of yellow fever virus.

It is felt, however, that monkey encephalitis induced by mouse virus should be much more carefully studied from the viewpoints of its cytology, and of the cellular relationship and distribution of the virus.

SUMMARY

1. A histological and cytological study has been made of an encephalitis of monkeys (*M. rhesus*) inoculated intracerebrally with the mouse strain of yellow fever virus.

2. The lesion in the monkey's brain is an acute, disseminated encephalomyelitis, extending apparently throughout the central nervous system, affecting the cellular tissues and causing necrosis of ganglion cells, both sensory and motor.

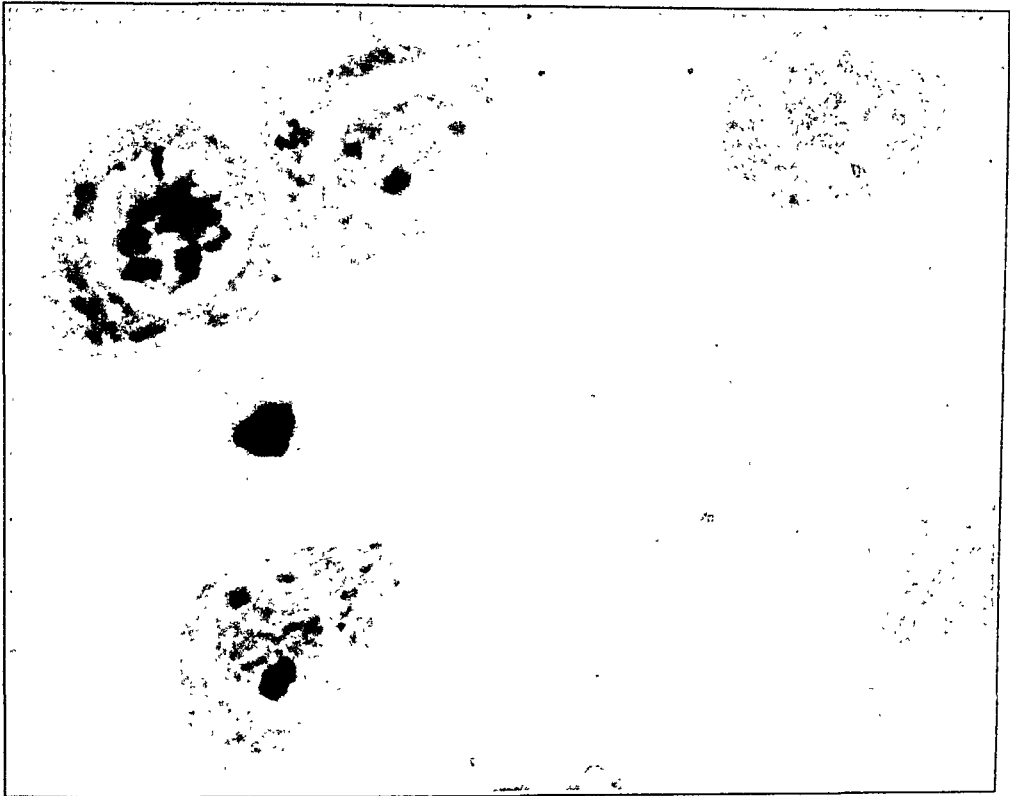
3. Intranuclear inclusions sometimes resembling, but more often differing from, those characteristic of yellow fever have been demonstrated in ganglion cells of the encephalitic monkey's brain.

4. On immunological and histological grounds it is judged that the virus of mouse and monkey encephalitis represents a biologically modified strain of yellow fever virus.

5. Cytologically the evidence of morphologically characteristic yellow fever intranuclear inclusions in the brains of encephalitic monkeys inoculated with the mouse virus is inconclusive.

PLATE 25

- FIG. 5. Monkey encephalitis to show phagocytosis of dead ganglion cell by polynuclear leucocytes.
- FIG. 6. Same showing diffuse polynuclear exudate.
- FIG. 7. Ganglion cell from monkey encephalitis showing oblong compact acidophilic intranuclear inclusions, at one end of which is a nucleolus. Note clear intranuclear space and aggregation about nuclear membrane of basophilic particles. Atypical of yellow fever.
- FIG. 8. Ganglion cell from monkey encephalitis showing nucleolus and acidophilic masses or inclusions, atypical of yellow fever.



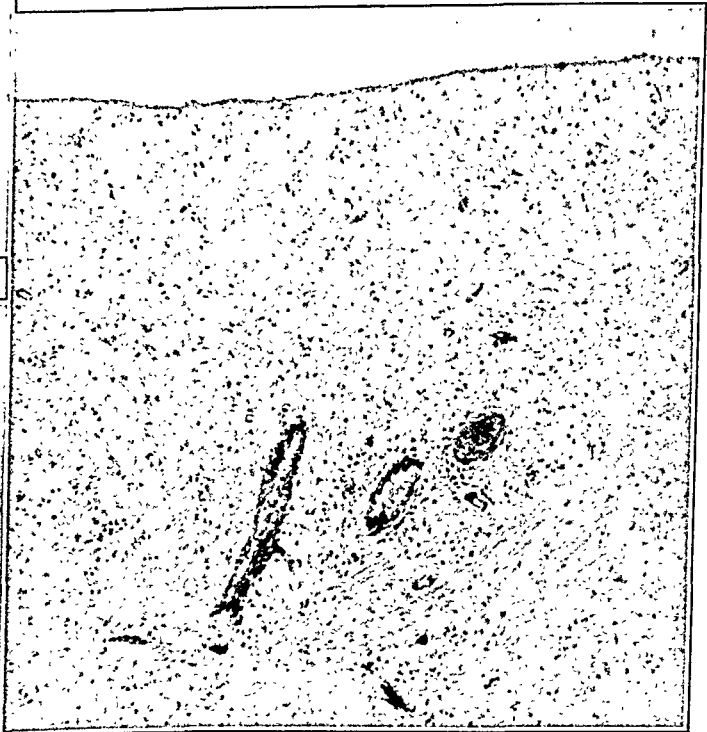
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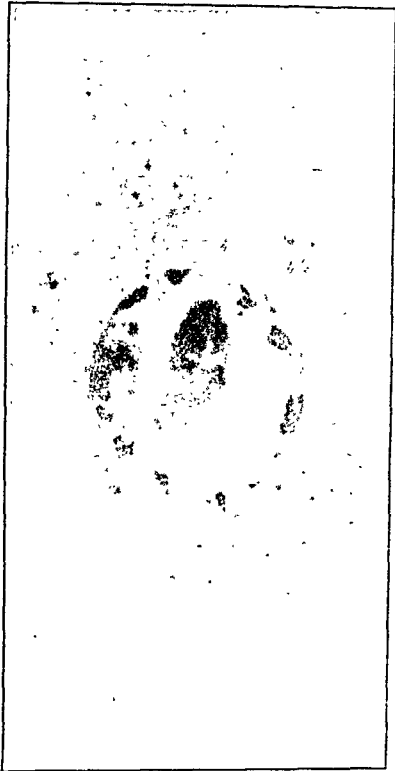
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PLATE 26

- FIG. 9. Monkey encephalitis. Phagocytosis of dead ganglion cell by mononuclear leucocytes.
- FIG. 10. Necrotic ganglion cells of monkey encephalitis. Central eosinophilic material filling nuclear space. Basophilic particles upon nuclear membrane. Atypical of yellow fever.
- FIG. 11. Large ganglion cell from monkey encephalitis showing large eosinophilic masses. The basophilic nucleolus is incorporated in the mass to the left. Atypical of yellow fever.



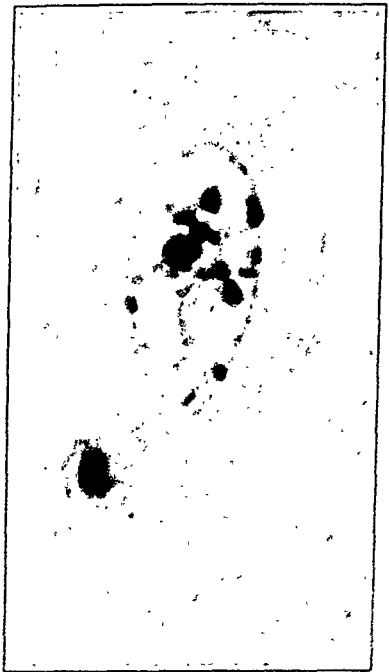
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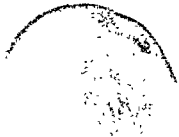


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Goodpasture



Yellow Fever Encephalitis of Monkey

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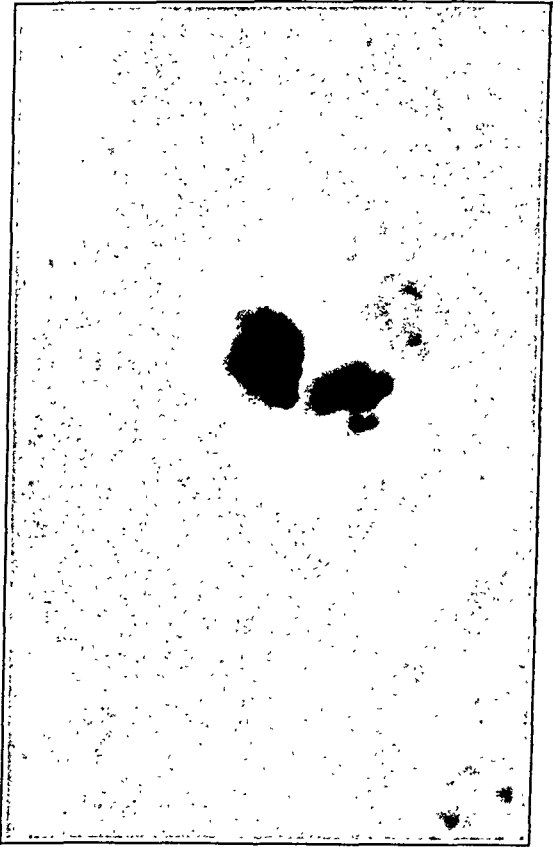


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Goodpasture



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Yellow Fever Encephalitis of Monkey

procedures, were stained with erythrosin-azur or phloxine-methylene blue.

The occurrence of intranuclear inclusions is indicated in the table, in the sites mentioned, by plus (+) signs. No inclusions were observed in other parts of the body. The minus (−) signs refer to negative observations and the zero (o) signs to instances where no

TABLE I

The Occurrence and Distribution of Intranuclear Inclusions in the Respiratory Tracts and Bile Ducts of 20 Monkeys.

Monkey No.	Disease	Nasal mucous membrane	Trachea	Bronchioles	Alveoli of lungs	Bile ducts of liver
5.	Poliomyelitis	−	o	−	+	−
12	"	+	o	−	−	−
34	Normal	−	−	−	+	−
38	Poliomyelitis	−	+	+	−	+
77	"	o	o	−	+	−
90	Measles	−	−	+	+	+
115	Diarrhea	+	−	−	−	−
116	Measles	−	−	−	+	−
123	"	−	−	−	+	−
135	Diarrhea	o	o	−	+	−
143	Poliomyelitis	o	−	−	+	−
156	"	o	−	−	+	−
164	Tuberculosis	−	+	−	−	−
168	Poliomyelitis	−	o	−	+	+
180	"	+	+	−	−	−
187	"	+	−	−	−	−
188	Diarrhea	+	−	−	−	−
195	Poliomyelitis	+	−	−	−	−
199	"	o	−	−	+	−
208	Diarrhea	−	o	−	+	−

tissue was available for study. Intranuclear inclusions were found in 20 out of the 60 monkeys. It is possible that still more might have been seen had serial sections on a large scale been examined. The distribution of inclusions was patchy. In only 1 monkey (No. 90) were they detected in 3 of the 5 locations listed in the table. In 2 others they were seen in 2 sites, while in the remaining 17 they were discovered only in 1 position. The locations in order of frequency were: alveoli of lungs (12), nasal mucous membrane (6), trachea and bile ducts (3 each), and bronchioles (2). It may be significant that inclusions were found in the bile ducts of the livers in the mon-

THE OCCURRENCE OF INTRANUCLEAR INCLUSIONS IN MONKEYS UNACCOMPANIED BY SPECIFIC SIGNS OF DISEASE *

W. P. COVELL, PH.D.

(From the Anatomical Laboratory, Washington University, St. Louis, Mo.)

Stewart and Rhoads ¹ in 1929 reported the discovery of intranuclear inclusions in the cells of the nasal mucous membrane of monkeys (*Macacus rhesus*) inoculated with the virus of poliomyelitis, but since they also found them occasionally in normal control monkeys they did not attach any particular significance to them. While making a detailed study of the lesions in experimental poliomyelitis I have encountered similar inclusions, not only in the situation mentioned by Stewart and Rhoads but, in addition, in the epithelial cells of the trachea, lungs and bile ducts. These intranuclear inclusions were likewise seen in animals which had not been subjected to the virus of poliomyelitis. I venture to report my findings briefly, because it is desirable to be as fully informed as possible concerning the presence of such inclusions unaccompanied by clinical symptoms in an animal like the monkey, which is used so much in the study of filterable viruses. The material on which this report is based was secured from 60 monkeys as follows:

Experimentally infected with poliomyelitis	37
Experimentally infected with nasal washings from cases of measles	5
Diarrhea	9
Generalized tuberculosis	2
Pneumonia	3
Normal	4

Autopsy was performed promptly after the animals died from disease or were killed with ether. Small pieces of the nasal mucous membrane, trachea, lungs, liver and other organs were fixed for twenty-four hours in Zenker's or Helly's fluid, and, after the usual

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ously a very incomplete impression of the details, for the color contrast which is so distinctive in original preparations is suppressed and the information to be secured by focusing is lacking.

Fig. 2 illustrates a multinucleated cell bordering the lumen of a bronchiole in each nucleus of which a typical inclusion is to be seen. One is thereby reminded of the appearance of intranuclear inclusions in chickenpox. The bronchioles which possess inclusions are often infiltrated with mononuclear leucocytes and in one instance a mite was found in close proximity, the tissue about it being inflamed.

The intranuclear inclusions discovered in the epithelial cells of the alveoli of the lungs, and in occasional cells free in the lumen, were of more striking appearance. One of them is shown at about the center of Fig. 3. The inclusion itself is roughly spherical, fairly compact and separated from the nuclear membrane by a marked halo. Such inclusions were more abundant in some lungs than in others. Though distributed in an irregular way, being more numerous in certain areas, they were not associated with detectable lesions, as in the other parts of the respiratory system.

Also in the intrahepatic bile ducts the inclusions were observed in the absence of distinctive tissue changes, other than repeated nuclear division (Fig. 4).

DISCUSSION

That these inclusions in the respiratory tract and bile ducts are caused by some virus of low virulence is a fair assumption, because despite repeated experiments by many investigators nuclear alterations of this kind have never been produced by agents other than viruses. Cole and Kuttner² go so far as to state in their paper on intranuclear inclusions in the salivary glands of guinea pigs: "It is true that Luger and Lauda have mentioned the occurrence of similar structures in a case of salvarsan dermatitis. Even though these lesions should be present in isolated instances of this kind, it would be necessary to demonstrate the absence of a filterable virus in the given instance before the present conception of the direct relationship between these nuclear changes and filterable viruses would become untenable." These investigators hold that the presence of a filterable virus is to be assumed when typical intranuclear inclusions occur, unless it is possible to prove the absence of a virus experimentally.

keys that also exhibited them somewhere in the respiratory tracts. However, seventeen monkeys, in which inclusions were noted in the respiratory tract, did not reveal them in the bile ducts. The number of positive observations is far too small on which to base any conclusions as to the primary site of action of the virus and its subsequent spread, if, as seems probable, a substance of this kind is involved. Neither does the number of animals examined permit any valid correlation between the type of disease and the incidence of inclusions as a possible accessory factor in rendering the tissue susceptible to the action of a hypothetical filterable virus. But it is to be noted that they were seen in 60 per cent of the animals experimentally infected with the measles virus, and in none of the animals which suffered from pneumonia.

The properties of the inclusions in the 5 locations were much the same. As seen in the nasal mucous membrane they corresponded in every particular with those previously reported by Stewart and Rhoads. They were most numerous in the more superficial epithelial cells, the nuclei of which were affected in varying degrees. In some, only a slight increase in nuclear acidophilic material was noticed, which of itself could not be regarded as noteworthy, but in many cells the process was carried to an extreme with: (1) characteristic margination of all basophilic material on the inner surface of the nuclear membrane, (2) clumping of the acidophilic material in the center of the nucleus, and (3) the appearance of a clear halo between the inclusion and the nuclear membrane. Such intranuclear inclusions were not seen scattered evenly throughout the extent of the mucous membrane, but were definitely restricted to a few small areas characterized by a necrosis and sloughing of the membrane, which in two cases was accompanied by an inflammatory reaction in the underlying tissues, including the glandular epithelium.

In the trachea, also, the inclusions were limited to similarly injured areas. An intranuclear inclusion in this situation is illustrated in Fig. 1. It is contained in a rather large nucleus slightly to the left of the center. At first sight it might be taken for an enlarged nucleolus, but careful examination showed that it was formed through the clumping together and fusion of acidophilic particles. Another nucleus just to the left is in an earlier stage of the reaction, being filled with finely divided acidophilic material. Photomicrographs like this, taken at a magnification of only 1500 diameters, give obvi-

yellow fever, which more closely resemble those that I have seen. At present no other intranuclear inclusions are known in monkeys, with which to make a comparison.

CONCLUSIONS

From these observations and those of Stewart and Rhoads, it seems likely that monkeys must now be listed with humans,³ guinea pigs,⁴ rats (Thompson⁹), rabbits (Rivers and Tillett¹⁰), and dogs (Cowdry and Scott¹¹) as animals in which one or more viruses sometime lurk, capable of producing intranuclear inclusions in the absence of recognizable clinical symptoms. Particularly is this of interest when monkeys are employed for experiments with viruses, which in the proper environments are definitely disease-provoking like those of poliomyelitis, yellow fever, chickenpox and measles.

If, then, we accept the inclusions as caused by virus action, the next question is whether we have to do with one virus spreading along the respiratory tract to all of the locations described, or with two viruses. This extension might be easily understood in the case of the respiratory tract, but how the bile ducts without corresponding alterations in intervening parts of the body come to be involved if only one virus is operating, is difficult to explain. I merely mention the possibilities without hazarding any interpretation. The indications are stronger for the operation of two viruses in man because the inclusions in visceral disease (VonGlahn and Pappenheimer³) are very different from those seen in the salivary glands (Goodpasture and Talbot⁴). Even in these cases we are not justified in reaching a definite conclusion as to the presence or absence of two viruses, because the difference in the resulting inclusions may be attributable to variations in the response of different types of cells to one and the same virus. The reason for making this qualification is that the sub-maxillary gland virus is known to produce enormous intranuclear inclusions with great nuclear hypertrophy in its usual site of action, namely, the ducts of the salivary glands of guinea pigs; and small inclusions with but slight increase in size, more closely resembling herpetic inclusions on intracerebral inoculation in guinea pigs.²

The nature of the virus or viruses, which leads to the development of the inclusions in the monkey described in this paper remains wholly unknown. The inclusions themselves are not for a moment to be compared with the other intranuclear inclusions in injured nerve cells in experimental poliomyelitis in monkeys, Covell,⁵ and the existence of which has been confirmed by Hurst.⁶ The latter are discretely rounded masses, which bear a resemblance to the inclusions pathognomonic of Borna disease.

The herpetic intranuclear inclusions produced in the liver cells of another species of monkey (*Cebus hypoleucus*) by Cowdry and Kitchen⁷ are much less dense in consistency and may fill the entire nucleus. The intranuclear inclusions caused by the virus of yellow fever in rhesus monkeys, likewise in liver cells (as noted by the same authors) are also different, in that they are laid down in clusters of discrete particles which do not typically fuse into a single mass. The difference may be due perhaps to the fact that different kinds of cells are responding, for Magalhães⁸ found intranuclear inclusions in the renal cells of monkeys experimentally infected with the virus of

DESCRIPTION OF PLATE

PLATE 27

Photomicrographs of intranuclear inclusions in the respiratory tract and bile ducts of monkeys taken at a magnification of 1500 diameters.

- FIG. 1. Type of intranuclear inclusion in the trachea. The cytoplasm of the cell is deeply stained. A compact, rounded, acidophilic mass is located centrally in the nucleus.
- FIG. 2. Intranuclear inclusions in the epithelium of a bronchiole. The nuclei have increased in number to form a giant cell with a tendency to margination of the basophilic chromatin. The inclusions are less compact than those in the alveolar epithelium and are in the form of discrete particles separated from the remaining chromatin by clear areas.
- FIG. 3. Intranuclear inclusions in the lung. Centrally located in the field is a nucleus of an alveolar epithelial cell containing an inclusion body. The monkey from which this photograph was made had received an intracerebral inoculation of poliomyelitis virus and was sacrificed during the early paralytic stage of the disease. The intranuclear inclusion is separated from the basophilic chromatin by a clear area.
- FIG. 4. Type of intranuclear inclusion found in the bile ducts of the liver. The basophilic chromatin is plastered against the nuclear membrane from which the inclusion is separated by a clear area. The similarity in the resemblance of this type of inclusion to that in the epithelium of the bronchioles is striking. The inclusions are in the form of clusters of discrete particles.

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3



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the obstruction is released, when the liver promptly becomes smaller and somewhat paler. This procedure deprives the liver cells of oxygen, subjects them to a considerable increase in pressure, interferes with their nutrition and permits the accumulation of metabolic products in the surrounding medium during the period of constriction.

Of the seventeen dogs used in these experiments, one died 4 hours after the operation in typical hypoglycemic convulsions, two were sacrificed after 24 hours, eleven after 48 hours, two after 72 hours, and one after 7 days.

The liver weight-body weight ratio in these animals was distinctly increased, the mean being 0.0376 ± 0.0035 , the individual ratios ranging from 0.025 to 0.047, only two being normal or below. Junkersdorf²⁰ found the liver weight-body weight ratio in normal dogs to be 0.030; Simonds and Brandes²¹ obtained a mean ratio of 0.0303 in thirty-one normal dogs. The mean ratio in these animals was, therefore, approximately 25 per cent higher than the normal.

The increase in the weight of the liver was due in part to edema. Simonds and Brandes²² observed an average increase of 2.5 times the normal outflow from the thoracic duct during mechanical constriction of the hepatic veins. On the basis of the microscopic examination of livers immediately, and 24 hours after constriction, it is assumed that much of this excess flow of lymph comes from the liver. The most marked change is in the lymphatics which surround the sublobular veins, many of which are encircled by widely dilated lymphatics filled with hyaline coagulated material (Fig. 1). These are apparently the radicles of the lymph vessels which follow the hepatic veins to the inferior vena cava, and thence along this vessel through the diaphragm into the posterior mediastinum. The connective tissue about these veins was rendered loose-meshed by accumulation of fluid between the cells. This can probably be accounted for as a result of damage to, and subsequent thrombosis of, the larger lymph vessels about the main branches of the hepatic veins. As shown by Opie²³ trauma is often an etiological factor in lymphatic thrombosis. The periportal connective tissue was also edematous.

Another element, of less importance, in the increase in weight of the livers of these dogs is the irregularly distributed increase of

ANATOMICAL CHANGES IN THE LIVERS OF DOGS FOLLOWING MECHANICAL CONSTRICTION OF THE HEPATIC VEINS *

J. P. SIMONDS, M.D., AND J. W. CALLAWAY, M.D.

*(From the Department of Pathology of Northwestern University Medical School,
Chicago, Ill.)*

This paper is a report of the changes observed in the livers of seventeen dogs whose hepatic veins were mechanically constricted for periods of 7 to 30 minutes for the purpose of studying the chemistry in the blood during the succeeding 24 to 72 hours. Practically all of the recorded anatomical studies of the liver following alterations in the hepatic circulation have been based upon permanent changes in the blood flow and, therefore, are concerned with more or less chronic modifications of that organ. Thus, the results of ligation of the hepatic artery have been investigated by Holst,¹ Behrend, Radasch and Kershner,² Ritter,³ Hori,⁴ Loeffler,⁵ and others. Bainbridge and Leathes,⁶ de Josselin de Jong,⁷ Rous and Larimore,⁸ Papilian,⁹ Chiari,¹⁰ and others, have studied the effect upon the liver of either ligation or thrombosis of the portal vein. Zimmerman and Hillsman¹¹ placed metal rings about the vena cava between the entrance of the hepatic veins and the heart. Hess,¹² in 1905, and more recently Satke¹³ and Saborowsky¹⁴ have reviewed the literature and discussed the results of obliterating endophlebitis of the hepatic veins. There are also occasional reports in the literature of alleged retrograde embolism of the hepatic veins (Heller,¹⁵ Risel,¹⁶ Meixner,¹⁷ and Reiniger¹⁸). But in all of the above experiments and observations the alteration in the hepatic circulation was continuous. We have been unable to find any studies of the changes in the liver resulting from a sudden and complete, but temporary, closure of the hepatic veins.

Mechanical constriction of the hepatic veins by the method described by Simonds and Brandes¹⁹ causes an immediate increase in the size of the liver, which becomes enormously distended with blood and dark brownish purple in color. This condition continues until

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and higher degree of specialization. The results of our experiments tend to confirm this view.

A variable number of central and sublobular veins are filled with clear structureless masses, some of which stain blue, others dark red (Fig. 4). As a rule, lobules whose central veins are thus occluded contain more blood than the adjacent lobules.

A characteristic finding in all of these animals is the presence of masses of cells within the sinusoids (Figs. 5 and 6). These cells are of three types: an occasional lymphocyte, a few polymorphonuclear leucocytes and a greater number of mononuclear cells with large round, oval or indented nuclei and moderately abundant cytoplasm. These latter cells appear to have originated from proliferation of sinusoidal endothelium. These cell masses are either small and compact and lie in an oval dilatation of the sinusoid, resembling those described by Simonds²⁵ and by Manwaring, French and Brill²⁶ in anaphylactic and peptone shock, or they are larger and more diffusely and loosely arranged in several adjacent sinusoids, but consist of the same cell types as the above. Within this second form of cell masses the cords of liver cells are disrupted and many of the included hepatic cells are swollen, stain with eosin and are without nuclei. These areas therefore have much in common with the focal necrosis described by Mallory²⁷ in typhoid fever.

Cell groups of the first type are most numerous in the dog that was allowed to live for 7 days and whose hepatic veins were constricted for 20 minutes. In many of these masses, especially in the 24 hour dogs, a red hyaline matrix is easily visible. These compact intrasinusoidal masses are probably of the same nature as those designated by Pearce²⁸ as conglutination thrombi. Their manner of formation is probably as follows. During the stagnation of the blood in the sinusoids, while the hepatic veins are constricted, a group of red cells becomes packed into a firm mass which cannot be broken up when the circulation is restored. These later fuse into a hyaline matrix in which is entangled an occasional lymphocyte and into which may wander a few polymorphonuclear leucocytes. The presence of this "foreign body" within the sinusoid stimulates the proliferation of the adjacent lining endothelium from which is derived the chief part of the cell content of the mass.

In the second type of cell mass there is no evidence of fusion of red cells. The presence of a group of necrotic hepatic cells may

blood. During constriction the amount of blood within the liver is enormous, but upon releasing the constriction most of the accumulated blood promptly escapes and when examined after 24 to 72 hours the liver as a whole is relatively poor in blood, with only a few scattered areas in which the central veins and adjacent sinusoids are distended with red cells.

On microscopic examination with low power one of the most striking features is the relative paleness of the central portion of the lobules (Fig. 2). The hepatic cells are swollen, more or less granular, many contain round clear spaces or vacuoles and some are without nuclei (Fig. 3). The vacuoles do not stain with osmic acid. The visible nuclei in this portion of the lobule vary greatly; some are swollen, extremely pale and washed out; others are compact and pyknotic, and relatively few are normal. The markedly swollen condition of these cells narrows, and, in places, practically obliterates the sinusoids so that the central part of the lobules is almost bloodless. At the periphery of the lobules is a zone of varying width in which the liver cells are more nearly normal. From this it appears that the hepatic cells in the central one-half or two-thirds of the lobule are less resistant to injury than those in the peripheral portion. A similar differential distribution of cell damage has been observed in other conditions, *e.g.*, chloroform poisoning, chronic passive hyperemia, and so on. In these conditions either a toxic agent or a disturbance of the circulation in the liver acts over a more or less long period of time. It has been suggested that the greater damage to the centrally located cells in the liver lobule is a result of their greater distance from the fresher part of the blood supply, the peripheral cells having the first opportunity to secure oxygen and nutriment from the blood as it percolates through the lobule, while the central cells receive only blood which has been depleted of substances essential to their life. But in our experiments the entire circulation through the liver was stopped temporarily. Hence both central and peripheral hepatic cells were subjected to identical conditions. The differential distribution of evidences of cell damage described above in the livers of our animals indicates a greater actual susceptibility to injury on the part of the cells in the central part of the lobules as compared with those of the periphery. Mallory²⁴ has suggested that the greater vulnerability of the central cells of the liver lobules is due to their greater functional activity

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serve to stimulate the proliferation of the sinusoidal endothelium. If this interpretation is correct, the process is the reverse of that described by Mallory ²⁷ as the probable pathogenesis of focal necrosis in typhoid fever.

In all these animals the Kupffer cells contained an abundance of brown, granular pigment resembling hemosiderin.

SUMMARY

The livers of dogs examined 24, 48 and 72 hours and 7 days after mechanical obstruction of the hepatic veins for periods of 7 to 30 minutes showed the following changes.

1. A mean increase of 25 per cent in the liver weight-body weight ratio, due to edema and to swelling of the hepatic cells.
2. Swelling, granulation, vacuolization and extensive necrosis of the hepatic cells in the central half or two-thirds of the liver lobules.
3. Marked dilatation of the perivascular lymphatics surrounding the sublobular veins.
4. The presence of hyaline thrombi in many central and sublobular veins.
5. Intrasinusoidal cell masses of two types: (1) small, compact, occluding masses probably originating in "conglutination thrombi" of red cells, and (2) larger, more diffuse and branching cell masses.
6. Hemosiderosis of Kupffer cells.

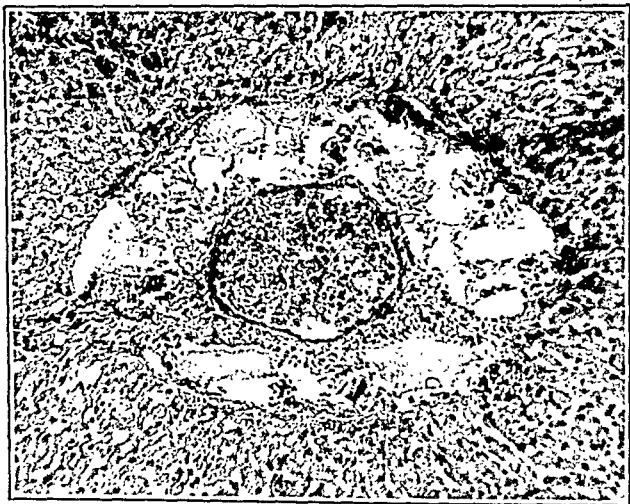
DESCRIPTION OF PLATE

PLATE 28

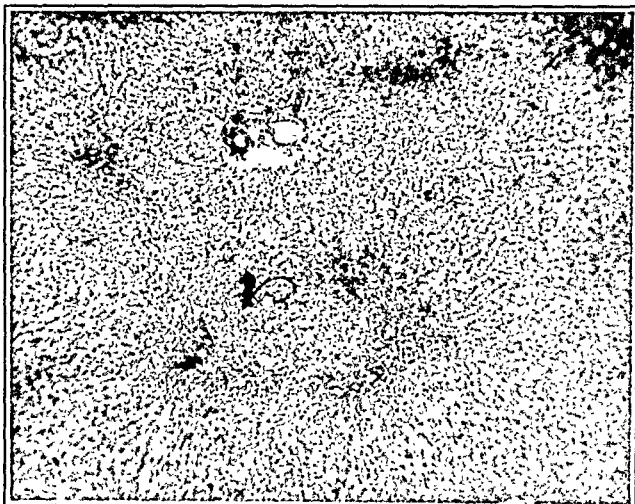
- FIG. 1. Distention of the perivascular lymphatics about a sublobular vein. $\times 100$.
- FIG. 2. Lower power field showing pale central portions of lobules. $\times 20$.
- FIG. 3. Swelling and necrosis of liver cells. $\times 325$.
- FIG. 4. Hyaline thrombus in central vein. $\times 200$.
- FIG. 5. Compact cell masses in sinusoids. $\times 160$.
- FIG. 6. More diffuse cell masses in sinusoids. $\times 200$.

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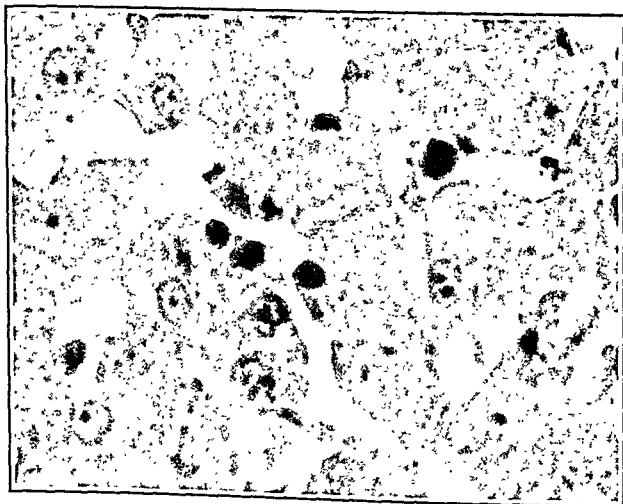




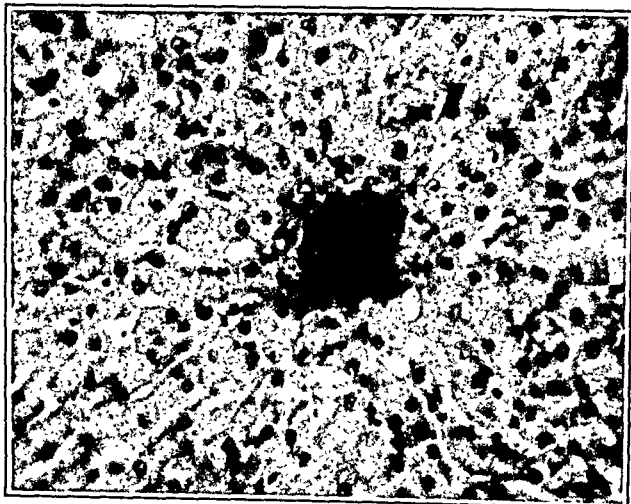
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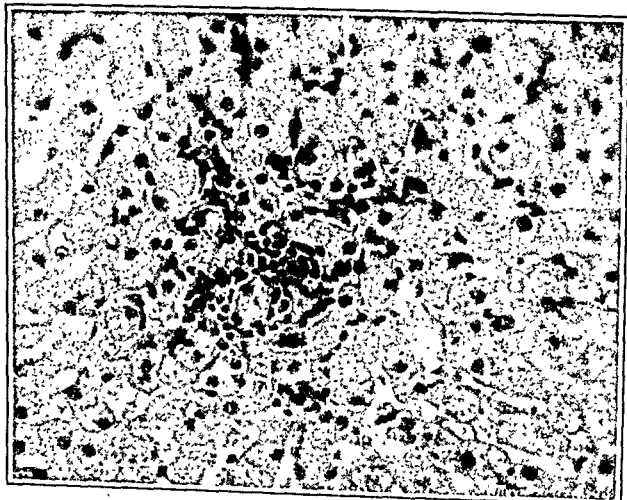
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material and stimulates a chronic inflammatory reaction in the meninges, which is apparently invariably fatal.

Since Stoddard and Cutler pointed out the necessity of bearing torula infection in mind in patients with increased intracranial pressure without localizing signs, chronic meningitis, or other obscure cerebral conditions, the number of recognized cases has steadily increased.

REPORT OF CASES

CASE 1. Mrs. F. M., 32 years of age, referred by Dr. Samuel Burrows, entered the Billings Hospital of the University of Chicago on Sept. 9, 1930, complaining of headache, pain and a feeling of tightness in the back of the neck, and pain behind the eyes. The family and marital history were unimportant.

Past History: In 1922, and again in 1928, the patient had pleurisy.

Present Illness: The patient's headache had been dull and inconstant for two years, but had become worse since the spring of 1929. A small lump in the right occipital region was noted in December 1929, from which a small cyst was removed in April 1930, and the bone scraped. The operation left a sinus which drained until June 1930, when at a second operation a small sequestrum was removed. On recovery from the ether anesthesia the headache, pain and feeling of tightness in the back of the neck developed and has persisted. Discomfort and a feeling of pressure behind the eyes has been noted for two months. During the two weeks before admission to the University Clinics nausea and vomiting occurred almost daily. The sinus in the occipital region has continued to drain.

Physical Examination: The patient was a rather thin, nervous woman, not acutely ill. In the right occipital region was a discharging sinus 2 mm. in diameter, which could be probed almost to the bone. Roentgen-ray examination showed no abnormalities of this area. The left maxillary antrum was cloudy and pus could be aspirated from it. Areas of increased density in both upper lung fields and marked enlargement of the mediastinal structures could be seen in roentgenograms, in spite of negative physical findings.

There was a slight stiffness of the neck but Kernig's sign was negative. Papilledema was $1\frac{1}{2}$ D on the right, and 1 D on the left with several exudative and hemorrhagic areas about the disk retinal margin on the left side. All of the other cranial nerves were normal. Sensation over the entire body was intact. All extremities were somewhat weak but the muscle tone was normal. The deep and superficial reflexes were normal, except for an absent right upper abdominal.

Course in Hospital: On admission to the hospital the temperature was 100° F, pulse 90, respirations 16, blood pressure 120/80; the white blood count was 9000, red blood count 5,100,000 and hemoglobin 90 per cent. The urine was normal and the Wassermann and Kahn tests on the blood were negative. On September 11, two hours after a rise in temperature to 101.6° F, the patient had a severe headache, vomited, talked irrationally and developed a generalized convulsion without focal signs; a second convulsion occurred thirty minutes later. The cerebrospinal fluid was clear and colorless with a pressure of 350 mm. of water and a cell count of 110 per cmm., of which 55 per cent were polymorphonuclears and 45 per cent lymphocytes. During the following five months the

TORULA INFECTION *

A REVIEW AND REPORT OF TWO CASES

JAMES W. WATTS, M.D.

*(From the Division of Neurology and Neurosurgery of the University Clinics,
University of Chicago, Chicago, Ill.)*

Our knowledge of yeast infections in man originated in 1894 when Busse discovered a yeast-like organism in a leg tumor which had been diagnosed sarcoma of the tibia. At autopsy he found similar tumor masses in various tissues, from which he isolated the organism. Thinking he had discovered the etiology of neoplasms he studied various other types of tumors and found the organism in one sarcoma and several nasal polyps, but the yeasts proved to be non-pathogenic. Two years later Gilchrist called attention to a type of dermatitis produced by blastomycetes. Sanfelice isolated torulae from human malignant tumors and these strains in the hands of Nichols proved pathogenic for rabbits and guinea pigs. In 1902 Frothingham studied a tumor-like mass in the lung of a horse caused by blastomycetes, which he placed in the subdivision of torula. It was not until 1916 when Stoddard and Cutler reviewed yeast infections in man that a real attempt was made to correlate the clinical and pathological features of the disease with the morphological and cultural characteristics of the organism producing it. Following Wolbach they classified these infections as (1) true yeast infections, (2) coccidioidal granuloma, (3) oidiomycosis, and (4) torula infection. They selected from the literature four cases without skin or subcutaneous tissue lesions, characterized clinically by cerebral symptoms throughout the course of the disease. To these they added two cases of their own and classified them as examples of torula infection. In this group fever and leukocytosis are not constant. The meninges and often the brain, lungs, liver and spleen are affected. The organism varies from 1 to 13 microns in diameter and has a deeply staining wall, which in the larger forms appears as a double line. In tissues it buds but does not sporulate; in cultures it buds but never produces mycelia. The yeast produces a gelatinous

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patient's progress was steadily downward. The temperature ranged from 100° to 103° F. There were numerous generalized convulsions, the papilledema increased to 5 D, nystagmus developed, and hypotonicity of the muscles of the extremities became marked. Drowsiness and mental confusion were outstanding. While in the hospital the occipital sinus was explored and the underlying bone and dura mater appeared normal. Later, ventriculograms disclosed a slight hydrocephalus but an otherwise normal ventricular system. In December Dr. Percival Bailey made a burr hole behind each mastoid process in the suboccipital region and probed the cerebellar hemispheres without finding pus. About two weeks before death, which occurred Jan. 29, 1930, retraction of the neck and a bilateral Kernig's sign were noted. The cerebrospinal fluid examinations are given in Table I.

AUTOPSY REPORT

The autopsy was performed by Dr. Paul Cannon five hours after death. There were about 50 cc. of a bloody fluid in the left pleural cavity and about 100 cc. in the right. Fibrous adhesions were present between the parietal and visceral pleurae at both apices but were more marked in the left, these being extremely dense. Otherwise the pleural surfaces were smooth except for small firm nodules 2 mm. to 4 mm. in diameter, surrounded by fibrous tissue, scattered uniformly throughout both upper lobes. The lungs were less crepitant than normal, especially posteriorly. The cut surface of the left lung showed numerous fibroblastic nodules scattered especially through the upper third of the upper lobe. There seemed to be no fresh areas of tuberculosis of either lobe. The right lung showed a dense fibroblastic tuberculosis extending through the medial third of the upper lobe. There was some fibrocaseous tuberculosis in the lower lobe.

The tracheobronchial lymph nodes were fibrocaseous. Some of the inferior glands on the right were 3 cm. in diameter.

The spleen weighed 400 gm. It was soft and was adherent to the left side of the diaphragm by fibrous adhesions. When sectioned, the surface was somewhat paler than normal and many tiny white nodules were present. These nodules were uniformly distributed throughout the pulp and were about 1 mm. in diameter.

Together the adrenals weighed 14 gm. The left adrenal was slightly larger and paler than normal. The cut surface showed the cortex to be pale. The right adrenal resembled the left in size and appearance. An occasional small scar was seen on stripping the capsule of the kidney. Otherwise the kidneys appeared normal.

TABLE I
Cerebrospinal Fluid in Case I

Date	Pressure	Cell count	Poly-morpho-nuclear leucocytes per cent	Lymphocytes per cent	Total protein mg.	Globulin	Sugar mg.	Chlorides as NaCl mg.	Lange	Culture	Wasser- mann
9/11/30	350	110	55	45	..	Trace
9/17/30	250	210
9/29/30	150	65	20	80	0	..
10/ 9/30	260	63	..	84 (16 l. m.)	85	Trace	Too low to read	758	..	0	..
10/20/30	Ventricle	14	30	70	54	..	20	691
10/23/30	400	24	16	84	..	Faint trace	5	674
11/10/30	320	43	27	73	108	Marked trace	15	674	1123211000	0	..
11/14/30	290	46	Trace	5555555421	0	Neg.
12/ 8/30	270	23	..	85 (15 degen.)	139	..	Too low to read	538	Neg.
12/30/30	Ventricle	20	50	30 (10 trans.) (10 l. m.)	0	Neg.
1/ 9/31	200	62	..	Too low to read	464	..	0	..
1/14/31	290	30	40	60
1/ 8/31	520	50	40	60

eosin, methylene blue or mucicarmine. However, moist sodium hydroxide preparations made from the spleen before fixation showed yeast cells, as well as many bacteria.

Adrenal: One adrenal has a large acute lesion with a necrotic center lying at the end just under the capsule. It is filled with organisms and in places where they are most numerous the parenchyma has completely disappeared and only the connective tissue trabeculae remain (Fig. 3). This area is surrounded by an exudate composed chiefly of plasma cells, with numerous lymphocytes and a few large mononuclear cells; fibroblastic and polymorphonuclear cells are rare. The cell columns just peripheral to the cellular reaction show slight evidence of compression in places. Budding forms are very numerous, many more than in the meninges (Figs. 3 and 6). Clear zones surround organisms in areas of necrotic tissue. In the same adrenal another acute lesion has formed around one of the central veins; the lumen of the vessel is filled with red blood cells, the wall densely infiltrated with torulae. Outside the vessel there are large numbers of organisms in necrotic tissue surrounded by a fibroblastic reaction similar to the other. The other adrenal contains a smaller, more acute lesion with very little tissue destruction and a small number of organisms. There is very little cellular reaction around it, but budding forms are very numerous.

Lungs: The lungs contain large dense scars with small, irregular, calcified foci. Surrounding them is fibrous tissue proliferation containing many fibroblastic nodules with giant cells and epithelial cells like those seen in the spleen. Some of the giant cells contain spaces with sharp borders, as if crystals had been dissolved out. Acute bronchitis and small areas of bronchopneumonia are scattered through the lungs. No yeast-like organisms or tubercle bacilli are seen.

Lymph Nodes: Most of the lymph nodes are completely replaced by fibrocaseous material with no active processes present. A large node near the hilum of the spleen is the seat of extensive hyaline degeneration and marked endothelial hypertrophy. There are occasional small tubercle-like structures with and without giant cells in the center, which in places are grouped together but separated by fibrous tissue. Yeast fungi could not be found, but some giant cells contain spaces resembling the residues of organisms like those in the spleen.

The mesenteric and retroperitoneal lymph nodes were normal in appearance. The lymph nodes at the hilum of the spleen were definitely enlarged and contained small, white, pin-head-sized bodies resembling tubercles. The lymph nodes surrounding the common duct were markedly enlarged and showed areas varying from 1 mm. to 8 mm. in diameter, which were whitish and opaque in appearance.

Brain and Meninges: The dura mater was under some increased tension. The cerebral hemispheres were of equal size, normal shape, and the convolutions were moderately flattened. The leptomeninges, which had a rather dry appearance, showed numerous small, white, tubercle-like nodules from 1 to 2 mm. in diameter scattered through them (Fig. 11); they were more numerous over the frontal lobes. The pia-arachnoid was thickened everywhere; this was most marked in the perichiasmal region where it was greatly thickened and had a gelatinous appearance. This exudate extended over the pons and medulla and over the superior part of the temporal poles. Around some of the larger vessels, particularly those of the Sylvian and Rolandic fissures, was a yellowish white exudate (Figs. 1 and 11).

Frontal sections of the cerebral hemispheres disclosed a slight degree of hydrocephalus but an otherwise normal ventricular system. The aqueduct of Sylvius was patent and the fourth ventricle normal. The prolongations of the pia mater between the gyri were thickened and that overlying the hippocampus, gyrus dentatus and insula was 2 to 3 mm. in thickness (Fig. 1). No cystic cavities were present in the cortex, the basal ganglia or elsewhere in the brain.

In sections passing just anterior to the temporal poles a small cavum septum pellucidum was seen. The cerebellum, pons and medulla were grossly normal, except for the exudate over their surface.

MICROSCOPIC EXAMINATION

Spleen: The spleen contains great numbers of tubercle-like nodules and shows marked hyaline degeneration which closely resembles amyloid but does not stain with Congo red. Giant cells are numerous, some having nuclei arranged around the periphery, others having the nuclei clumped in one part of the cytoplasm. No organisms can be found, but the giant cells occasionally show spaces resembling the residues of digested organisms; distinct outlines or shells appear clumped together in a clear space. No double contours can be seen, neither does this débris stain with hematoxylin-

The granuloma covering the corpora quadrigemina, which is 6 or 7 mm. thick, is composed largely of necrotic material resembling caseation in some areas. The only cells not having degenerative changes are in immediate proximity to the blood vessels. They are chiefly lymphocytes, but polymorphonuclear leukocytes, plasma cells and large mononuclear exudate cells are numerous. Often a vessel will have a thickened wall almost surrounded by organisms, which are surrounded by a thick collar of lymphocytes or leukocytes with degenerated areas peripheral to this.

Most of the blood vessels show proliferation of the adventitia and often contain torulae within their walls; some have groups of torulae in their walls not surrounded by inflammatory cells. Several large vessels present marked endarteritis, the lumen being almost obliterated by a proliferation of endothelium. Torulae are found in the intima of several of these vessels. One space lined with flattened, elongated cells resembling endothelium is filled with organisms.

The ganglion cells of the cerebral cortex are in various stages of degeneration; most of them are swollen and distorted, with a complete chromatolysis of the Nissl substance, and many appear as faint outlines. The astrocytes are increased both in size and in number. The myelin sheaths in the subcortical white matter are greatly thinned out. They are swollen, fragmented, and in places small vacuoles are present. A normal number of myelin sheaths enter the cortex but they are swollen, beaded in appearance and lack continuity. Many mucocytes occur throughout the white matter. The nerve fibers in the white matter are severely broken up, the fragments are beaded and often irregularly shaped globules are the only remnants. There is no fatty degeneration, except in the perivascular inflammatory cells.

Ganglion cells, myelin sheaths and nerve fibers in the corpus striatum show changes similar to those in the cortex. No fatty degeneration is present. The lower part of the lenticular nucleus, adjacent to the granuloma described above, has many dilated blood vessels with marked perivascular, lymphocytic and leukocytic infiltration. Many inflammatory cells are also found free in the tissue, the polymorphonuclears predominating. The ependyma is much thickened and underlying it is a marked proliferation of glia which produces a protrusion of the ependymal lining into villus-like structures (granular ependymitis).

Kidney: The epithelium of the kidney is swollen, granular and in places necrotic.

The other organs contained nothing of interest.

Brain and Meninges: Sections for microscopic study were taken from the cerebral hemispheres, corpus striatum, midbrain, pons and cerebellum. They were stained with thionin, mucicarmin and scarlet red. Myelin sheath stains were made after the method of Weil, and nerve fiber stains after Bielschowsky. In addition the meninges were stained with hematoxylin and eosin, with carbol-fuchsin for tubercle bacilli, and by Van Gieson's method for connective tissue.

The leptomeninges over the convolutions are slightly thickened and infiltrated with plasma cells. Wherever there is a blood vessel a marked cellular reaction is present around it, and where numerous blood vessels occur close together a granuloma is formed in the meninges. Over the convexity of the brain, where the reaction is less marked, degenerative changes are few; plasma cells predominate and there are many cells with large, oval or elongated, pale staining nuclei and lymphocytes. Giant cells are scattered through the meninges, most often forming the center of a nodule. Considerable connective tissue is shown by the Van Gieson method. Many fat globules can be seen in scarlet red preparations, usually within cells in proximity to areas of necrosis, but not within the areas of marked necrosis.

There is a granuloma at the base of the corpus striatum, arising in the meninges and extending a considerable distance into the brain. In this area are many giant cells and numerous areas of complete degeneration. There are many lymphocytes and polymorphonuclear leukocytes, the former predominating; plasma cells and cells with large, pale, oval or elongated nuclei are fewer than over the convexity of the hemispheres. There is much rather old connective tissue and many new blood vessels in the granuloma. Torulae are scattered through the meninges, often occurring in large groups without evidence of cellular reaction around them (Figs. 4 and 5). The adjacent area of the brain has many dilated blood vessels with marked perivascular lymphocytic and leukocytic infiltration, and many inflammatory cells free in the tissues, of which the polymorphonuclears predominate. Pial funnels greatly thickened by a lymphocytic reaction dip far into the brain.

numerous torulae, plasma cells and lymphocytes. The other area of softening is different in many respects. Several blood vessels with a lymphocytic infiltration about them enter it. The cellular reaction is composed largely of glia and cells with pale elongated nuclei; polymorphonuclear and plasma cells are rare and no torulae are present. A long, narrow zone of degeneration extending from the cortex centrally through the white matter is revealed by the presence of many fat globules, most of which are within cells.

In one area in the dentate nucleus are numerous groups of small, densely and evenly staining round bodies from pin-point size to 2 microns in diameter, which stain purple with thionin. They are usually discrete but often appear to be budding. They generally occur in double file along a straight or sinuous course, and the flattened endothelial cells of a capillary can usually be identified along the route. They are most probably minute torulae within capillaries.

The Organism: Various stains were used and although the organisms took many of them well, they were difficult to identify in the tissues unless present in large numbers. In looking for a differential stain, those for mucin were considered because of the gelatinous substance produced by torula in the meninges. Bailey and Schaltenbrand have demonstrated that the clear, non-staining substance which occurs in acute swelling of oligodendroglia is in reality a mucin-like substance. This was shown by Grinker and Stevens to be a specific type of regressive change found in no other glia, and represents the same process as mucoid degeneration. Torula stains the same color with mucicarmin, but the morphology is so different that no confusion will arise in the differentiation from mucocytes, although the latter are numerous in the brain in this case. By this method the organisms can be easily found with the low power objective of the microscope: they are pink, the cell nuclei brown and cell cytoplasm yellow. Goto obtained a somewhat similar result using Best's carmin stain.

Stained by this method the organism under the lower power of the microscope is a round or oval, somewhat refractile body with a pale staining center and a deeply staining pink wall. Under the oil immersion objective the small forms have a homogeneous center and a single line composing the wall. Often the medium sized and large forms have a double-contoured wall. When the inner one is brought

The nuclei of the pons and midbrain are in fairly good condition. Some of the ganglion cells are slightly swollen and others have a somewhat sclerotic appearance. The myelin sheaths are less damaged than in the subcortical white matter. Mucocytes, which are pink-stained with mucicarmin, are numerous. There is a triangular area of incomplete softening with the base on the surface of the pons and the apex inside. It is filled with large scavenger cells and invaded by many new blood vessels. The border line is sharp. The parenchymatous elements about the periphery are fairly well preserved and the microglia are increased. Myelin has completely disappeared from this infarct. The subarachnoid space surrounding the midbrain and pons is completely obliterated.

Complete chromatolysis of Nissl's substance has occurred in the ganglion cells of the dentate nucleus. Throughout the granular layer of the cerebellum are small foci where these cells have dropped out. All of the Purkinje cells show degenerative changes; many are rounded or distorted and appear as faint outlines or shadows. In one area there is a complete falling out of the granular and Purkinje cell layers of one-half of a folium and the adjacent half of the neighboring folium. In this area the glia are somewhat increased. The scarlet red stain discloses many fat globules in the cortex of the adjacent halves of the two folia, extending centrally as far as the granular layer, with only an occasional droplet within it. The myelin sheaths in the white matter are greatly thinned out; they are swollen, fragmented, and in places small vacuoles are present. The myelinated fibers entering the folia are sparse and no fine fibers can be seen entering the granular layer. Using Bielschowsky's method, the nerve fibers in the cortex of the cerebellum are seen to be fairly well preserved. In the white matter they are markedly fragmented, and the fragments are beaded or appear as irregularly shaped globules.

There are two areas of softening about 1 mm. in diameter, one being in among the ganglion cells of the dentate nucleus, and the other in the white matter enclosed by the arms of the nucleus. The latter has in it several large giant cells containing torulae; these lie in the midst of a large collection of polymorphonuclear leukocytes which are surrounded by a thick ring of cells with large, oval, pale nuclei. Scattered through this lesion are similar pale nuclei which have assumed a more rounded or a more elongated form, as well as

sharply into focus the outer appears as a less deeply staining, refractile ring. Pink spicules radially arranged, with a broad base attached to the outer wall of the organism, and a pointed end are present on many of them (Fig. 5). In the tissues these yeast-like bodies are often surrounded by a wide clear zone, especially when in groups or in tissue undergoing regressive changes (Figs. 3, 5 and 6). The distal ends of the spicules usually extend out to the periphery of this clear zone but have not been seen to reach beyond it. This effect is most probably due to the action of the fixative.

The centers of the medium sized and large forms have several variations; some are homogeneous like the small ones; others are homogeneous except for the presence of two to five irregularly shaped, clear spaces, or non-staining inclusions. Many, however, have the entire portion within the wall composed of a tan, flocculent material (Figs. 5 and 6). This may be evenly distributed; it may contain in it clear spaces, or when small in amount form a ring just medial to and touching the inner wall of the organism.

Often a black, chromatin-like substance lies within the organism, which gives it the appearance of having a nucleus.

Most of the torulae occur free in the tissues but are sometimes found within giant cells (Fig. 13). Here they vary much in the depth of the stain taken. Some are deep pink, others in the same giant cell are colorless. There can be no doubt about the identity of the latter because of their characteristic size and shape, and some even have a double-contoured wall. This is important to note, because the inclusions in the giant cells of the spleen, lung and perisplenic lymph node may be of this nature, though it is true that no double-contoured forms are seen.

Reproduction is by budding, occurring usually in medium sized and large forms (Figs. 3, 5, 6 and 7). All stages are seen from a small bud-like projection from the circumference of the organism to dumb-bell forms where the mother and daughter cells are of equal size. Finally the two bodies separate, though occasionally they continue to be connected by a pink-staining band. Usually the daughter cell breaks off when it reaches one-half to two-thirds the size of its parent.

Budding forms are numerous in the lesions in the adrenals, much less frequent in the meninges. No budding has been seen in

TABLE II
Cultures of Yeast-Like Organisms from Spleen*
Sugar Concentration as Indicated

Strains	1% Dextrose	0.5% Fructose	0.5% Galactose	0.5% Inulin	0.5% Lactose	0.5% Mannose	1% Sucrose	0.5% Maltose	0.5% Xylose
Non-pigmented	3	SI ⊕ (1)	SI + (1)
	4	SI ⊕ (1)	SI + (1)
	6	SI ⊕ (1)	SI + (1)
Pigmented	7	SI + (7)
	Torula rosa	SI + (14)	SI + (14)	SI + (14)
Known stock ..	Saccharomyces cerviciae	⊕ (1)	SI + (1)	SI ⊕ (1)	SI ⊕ (1)	SI + (14)
	Oidium lactis
	Blastomycetes dermatitis
Sugar Concentration of 2.5 Per Cent									
Strains	Dextrose	Fructose	Galactose	Inulin	Lactose	Mannose	Sucrose	Maltose	Xylose
Non-pigmented	3	⊕ (2)	⊕ (3)
	4	⊕ (2)	⊕ (3)
	6	⊕ (2)	⊕ (3)
Pigmented	7	SI + (7)	SI + (14)
	Torula rosa	SI + (7)	SI + (7)	SI + (12)
Known stock ..	Saccharomyces cerviciae	⊕ (2)	+ (3)	⊕ (3)	+ (2)	⊕ (2)

* + = acid ⊕ = acid and gas SI + = slight acid SI ⊕ = slight acid, one bubble of gas () = figure in parentheses represents day reaction first appeared

the culture ages. The colonies spread slightly. There is a variation in the size and shape of the cells. Budding forms are numerous in young cultures. With carbohydrate concentration of 2.5 per cent, dextrose was fermented on the seventh day with production of acid without gas. Fructose and mannose showed a slight amount of acid on the twelfth and fourteenth days respectively. Neither acid nor gas was formed in any of the other sugars tested (Table II). With 0.5 per cent concentration of carbohydrate similar reactions were obtained, except that fermentation of dextrose did not appear until the fourteenth day.

Animal Pathogenicity: Rats and mice were inoculated intraperitoneally with pure cultures of each of these strains. The mice died during the third week after inoculation. Autopsies were performed and cultures made from the tissues but no yeast-like organisms were recovered.

The rats remained well and were sacrificed one month after inoculation. The rats inoculated with the strains producing no pigment (*a*) were normal so far as could be determined, and cultures made from the liver, spleen and kidneys were sterile. The rats inoculated with the strain producing yellow pigment (*b*) showed white patches on the spleen and a few on the liver. These white patches were similar to those described by Tanner and Dack in rats inoculated with yeasts isolated from sore throats, but yeast could not be isolated in cultures of these organs. Histological sections of the spleen contained nothing which could be definitely identified as a yeast.

Two guinea pigs were injected in the groin with cerebrospinal fluid Jan. 10, 1931, a short time before the patient's death. One died February 19, and autopsy revealed exudate in both pleural cavities and some consolidation of the lungs. Some elements in the microscopic sections might be considered yeast, but none could be cultured from this exudate or any of the other viscera, though bacteria were numerous. The second pig, which had been in good health, was killed April 18 and cultures made on Sabouraud's medium, Heinaman's potato medium and in dextrose broth from the brain, liver, spleen, kidney, lung, peritoneal cavity and heart's blood. All organs appeared normal. Pure cultures of a budding, yeast-like organism were recovered from lung and kidney. These organisms were oval and stained very faintly with mucicarmin, but a deep

giant cells; the colorless zone surrounding the bodies, so frequently present in the extracellular ones, is occasionally found here. No spores, mycelia or hyphae are seen.

BACTERIOLOGICAL EXAMINATION *

Method of Isolation: Moist sodium hydroxide preparations of the spleen showed under the microscope yeast cells, as well as many bacteria. Small blocks were removed aseptically from the tissue and streaked on the following media: Sabouraud's dextrose agar plates, carrot cylinders, Corper's glycerinated potato cylinders, Petroff's, and Hohn's egg medium. The cultures were made in duplicate, one set being incubated at room temperature and the other at 37° C.

A part of the tissue was ground in a sterile mortar and emulsified in normal saline. One half of this was heated to 53° C for twenty minutes to kill off the bacteria. Two rats and two mice were then inoculated with each emulsion. The mice died with evidence of bacterial infection, but no yeast-like organisms were recovered. The rats remained normal.

Yeast-like organisms grew out in Sabouraud's agar, carrot cylinders and the egg medium of Petroff and of Hohn (see Húth). By repeated subculture on Sabouraud's agar, pure cultures were obtained from the carrot cylinder. Two varieties of yeast were isolated, one producing no pigment and one producing a yellow pigment.

Cultural Characteristics: (a) *Strain Producing No Pigment:* On Sabouraud's agar the growth is white and shiny. The colonies are discrete, spreading slightly with age (Fig. 10). The cells are round and quite uniform in size and shape. Budding forms are quite numerous in young cultures. Fermentation tests were made using both 0.5 per cent and 2.5 per cent concentrations of the carbohydrates. The higher concentration of sugar seemed to hasten the reaction and cause a definite amount of gas to be formed in some of the sugars (Table II). In this, acid and gas were formed in dextrose, fructose and mannose. There was no reaction in galactose, inulin, lactose, sucrose, maltose or xylose at the end of fourteen days.

(b) *Strain Producing Yellow Pigment:* On Sabouraud's agar the growth is a light yellow at first, becoming more deeply pigmented as

* I was assisted in the bacteriological work by Miss Elizabeth Petran and Miss Bertha Kaplan of the Department of Hygiene and Bacteriology.

to speak, but pointing to the right side of her chest as though it hurt. The temperature was 101.8° F, pulse 140, respirations 48. The teeth were in poor condition, mouth dirty, tongue parched, lips covered with sores and breath foul. She could close her mouth voluntarily but seemed unable to keep it closed. The chest was resonant throughout but the breath sounds could barely be heard over the right lung. The heart sounds were clear and distinct at the apex but distant elsewhere; the heart did not appear enlarged. Ophthalmoscopically the blood vessels were moderately engorged, disk margins blurred and there was slight papilledema. Pupils were equal, reacted to light and accommodation and the extraocular movements were normal. No facial weakness. The tongue could be protruded only a short distance but it appeared in the midline. All extremities were rather weak but there was no paralysis. A coarse, slow tremor of the hands was present and the muscles of the arms were rather stiff. Sensation and reflexes in upper and lower extremities were normal. A bilateral unsustained ankle clonus was obtained. There was a little stiffness of the neck and a slightly positive Kernig's sign.

Laboratory Data: A lumbar puncture was made and 96 cells found, 84 of which were lymphocytes and 12 polymorphonuclear leukocytes. A smear was made for tubercle bacilli but none was found. A culture of the fluid yielded *Staphylococcus aureus* and a diphtheroid bacillus.

Course in Hospital: Four days after admission her condition grew rapidly worse, the extremities became cold and cyanotic and large bubbling râles could be heard in the chest. Postmortem roentgenogram of the chest showed an extensive pneumothorax on the right side.

AUTOPSY REPORT

The autopsy was performed by Drs. E. D. Peasley, J. W. Budd and C. F. Obermann thirteen hours after death.

The only significant findings were in the lungs and the central nervous system. The left pleural cavity contained a small amount of clear, straw-colored fluid. When the right pleura was punctured a considerable amount of foul-smelling gas escaped under pressure. About 400 cc. of grayish, purulent fluid was present and the lung had collapsed so that it occupied about one-fifth of the cavity. An abscess 1 cm. in diameter could be seen just beneath the pleura near the base of the inferior lobe of the right lung on the anterior lateral aspect. The bronchi contained mucopurulent exudate which completely occluded many of them; there was marked bronchitis and tracheitis. The left lung appeared normal, except for a few healed lesions in the serosa.

Brain and Meninges: The leptomeninges of the brain were thickened and had a milky, grayish appearance, most marked around the interpeduncular region and fissure of Sylvius. The blood vessels were markedly congested. The convolutions were normal.

violet with the Gram-Weigert stain. These were transferred to 2.5 per cent dextrose, lactose, sucrose, maltose, mannose, galactose, inulin, fructose and xylose; acid and gas formed in dextrose, mannose, and fructose in twenty-four hours, but no change appeared in the others in two weeks. These cultures were also put into 1 per cent dextrose, lactose and saccharose, and 0.5 per cent maltose, mannose, inulin, xylose, fructose, rhamnose, raffinose, ducitol and galactose, with the formation of acid and gas within twenty-four hours in the dextrose and fructose. No reaction appeared in the other tubes on prolonged standing. Cultures from the lung and the kidney produced identical reactions. Torulae were not found in microscopic sections of lung, kidney or other organs. This strain was translucent to white, at first becoming brownish with age (Fig. 12). It fermented the same sugars as the pigment-producing one from the spleen. On April 24, two guinea pigs and two rats were injected intraperitoneally with cultures from the lung. One of the rats died September 11, the other animals were sacrificed the same day and cultures made of the brain, lungs, kidneys and spleen on Heinaman's potato medium and in dextrose broth. An oval, budding organism morphologically like the one injected was recovered from the brain and spleen of the rat that died, and from the spleen of one of the guinea pigs. All of the tissues appeared normal.

CASE 2. * Mrs. F. H., aged 48 years, entered the University of Iowa Hospital, Feb. 20, 1929, complaining of headache, weakness and coldness of the extremities, inability to speak, and difficulty in swallowing. Her family history was of interest, in that one child died of meningitis. The patient had pneumonia in 1925.

Present Illness: Began Nov. 15, 1928, three months before entering the hospital, with headache, pain in back of the neck and vomiting. Five days later she complained of weakness and coldness of the extremities, more on the right than the left. November 22 she became unable to speak and attempts to eat produced attacks of strangling and coughing. This continued and she was unable to take anything but fluid after this symptom appeared, and consequently lost 70 pounds in weight. About one week before admission to the hospital her husband noticed she was acting queerly; she would sit huddled up in bed with her mouth hanging open, apparently unable to keep it closed. All of her symptoms continued and she was brought to the hospital.

Physical Examination: Showed a markedly emaciated, white woman, sitting doubled up in bed, mourning and shaking her head, mouth hanging open, unable

* Through the courtesy of Drs. G. H. Hansmann, C. Van Epps, and C. F. Obermann of Iowa City I have been able to study this case, which has features not present in my own.

a halo. Around a large cavity are often numerous small cavities, each containing one or more organisms. The organisms are very large, often double-contoured with rare budding forms (Fig. 15). Numerous convexoconcave and biconcave forms, like those shown by Freeman in his comparative study of this condition, are seen. Other forms have three concavities visible. Several cysts are seen which contain a moderate sized blood vessel; in all of these, and in a few others where no vessel can be identified, there is some round cell reaction.

The spinal meninges resemble those of the brain except that the organisms are fewer. No lesions are found in the spinal cord.

Cultures were made at autopsy with the following results: (a) blood: *Streptococcus hemolyticus*; (b) empyema: a yeast-like organism, a streptococcus, and *B. proteus*; (c) lung abscess: *Staphylococcus aureus*, a streptococcus, and *B. proteus*; and (d) brain: *Streptococcus hemolyticus* and *B. proteus*. Unfortunately the yeast grown from the pleural exudate was not saved, so no studies were made of it.

DISCUSSION

Torula is distributed widely in nature, having been cultivated from wasp nests, the stems of many plants and grasses, the bodies of numerous insects, and from pickle bran (Buchanan, Duggar, Stevens). It has been found in milk (Klein) and in canned butter (Rogers). It seems that all types are originally non-pathogenic, becoming pathogenic only under suitable conditions. The low pathogenicity is borne out clinically by the fact that most cases are recognized only when they attack the central nervous system.

Freeman divided the lesions of the brain into three general orders: meningeal, perivascular and embolic. The perivascular is frequently associated with the meningeal. Except in Ball's Case 1, meningitis was present in all of those with cystic cavities, which are considered by Freeman to be embolic phenomena. Cystic cavities were reported by Ball, Benda, Bettin, Flu and Woensdregt, Freeman and Weidman, von Hanseemann, Pierson, Rusk and Farnell, Smith and Crawford, Stoddard and Cutler, Stone and Sturdivant, Weil, White, and Williams. Cases with meningitis without cysts were recorded by Evans, Goto, Hall and Hirsh and Mock, Hansmann, Hirsh and Coleman, Lynch and Rose, Massee and Rooney, McKendree and

The brain was cut into thin coronal sections which revealed numerous cystic cavities containing a gelatinous material. They were found (1) in the basal ganglia of both hemispheres, varying in size from 1 to 8 mm. in diameter (Fig. 2); (2) the right cerebellar hemisphere at about the dentate nucleus, 1 to 3 mm. in diameter (Fig. 14); (3) the cortex of the right frontal lobe on its ventral aspect, and (4) the pons in the vicinity of the substantia nigra. There was moderate hydrocephalus.

The meninges of the spinal cord were thickened and the blood vessels were congested. Several white, flaky patches were seen in the meninges. The cord appeared normal externally and on section.

MICROSCOPIC EXAMINATION

Lung: Microscopically the greatest changes are in the right lower lobe of the lung, where there is an extensive pneumonic process of the lobular type. Between the patches of pneumonia are many large abscesses filled with polymorphonuclear leukocytes with relatively little fibroblastic reaction. Several large foreign body giant cells are present, but no yeast organisms can be found although they were isolated by culture from the pleural exudate.

Meninges: Microscopically there is a chronic inflammatory process in the meninges. The exudate is made up largely of lymphocytes and plasma cells, with a few polymorphonuclear leukocytes. Giant cells are quite numerous; the nuclei in some are arranged about the periphery and in others are grouped together in the center of the cell, varying from ten to twenty in number. Torulae are found scattered through the meninges and within giant cells; they vary considerably in size. Budding forms are seen occasionally. Perivascular reaction is much less prominent than in Case 1.

The cysts have a characteristic appearance (Fig. 9). A typical one appears as a cavity with practically no reaction around it. Often it is multilocular, being divided into compartments by strands of necrotic brain tissue running from one wall to another. The wall in many of the cysts appears compressed, but not sufficiently to account for its size by expansion alone, due to the material produced by the organism. Large numbers of organisms lying free and in necrotic material near the periphery are present. Those lying in areas of necrotic material are surrounded by clear zones resembling

The finding of torulae in the gasserian ganglion in four cases (Hall, Hirsh and Mock, Hirsh and Coleman, and Semerak (two cases)) suggests direct extension from the pharyngeal structures. Although most cases have been reported as meningo-encephalitis, and a few as localized abscesses (Brewer and Wood, McGehee and Michelson) and infections of the nasopharynx, many were generalized (Hall, Hirsh and Mock, Pierson, Rappaport and Kaplan, Rusk and Farnell, Sheppe, Stone and Sturdivant, Versé, White, Williams, and Watts' Case 1). After the central nervous system, the lung, spleen, kidney and adrenal each contain the organisms most frequently. In contrast to oidiomycosis and coccidioidal granuloma, a skin lesion has been present in only one case (Rappaport and Kaplan) and a bone lesion in one (Brewer and Wood). In the latter the spinous processes and laminae were eroded but no torulae were found in the bone itself.

Dissemination from the respiratory tract may be by the blood stream or lymphatics. Organisms in the gasserian ganglion of four cases suggest lymphogenous infection. Spitzer's experiments support this view; he produced an ascending inflammatory neuritis spreading up to and implicating the gasserian ganglion by injecting *Abrus precatorius* into the dental pulp of dogs. The infarct in the pons, torulae in the intima of meningeal vessels producing an endarteritis, and minute torulae in capillaries of the cerebellum of Case 1 add weight to the theory that cystic cavities in the deep white, or gray matter of the brain are embolic phenomena. Here by embolic phenomenon is meant not the occlusion of a small vessel with resulting ischemic liquefaction, but the passage of torulae by the blood stream and the deposition with the production of a cyst by the organisms, by lysis, or by expansion.

The cerebrospinal fluid findings are what one would expect in chronic meningitis. The sugar has been reduced in all cases in which it was noted to about the same degree found in tuberculous meningitis, as shown in a review by Watts and Viets. The chloride content, measured as sodium chloride, was reduced to the unusually low level of 464 mg. per 100 cc. This is even lower than occurs in tuberculous meningitis, and Fremont-Smith in his comprehensive review records no figure as low. The colloidal gold curve has been noted in very few instances, but in three it was distinctly paretic in type (Hansmann, Wortis and Wightman, and Watts). Several instances of the non-specific character of the curve are given by Watts and

Cornwall, Rappaport and Kaplan, Shapiro and Neal, Semerak (two cases), Sheppe, Stoddard and Cutler, Swift and Bull, Türck, Versé, Wilhelmj, Wortis and Wightman. Case 1 of this report belongs to the latter group and Case 2 to the former. In five, the diagnosis of meningitis was made by detecting the organism in the cerebrospinal fluid (Evans (two cases), Lynch and Rose, Shapiro and Neal, Wortis and Wightman), an autopsy not having been performed. Stoddard and Cutler, and Freeman in his comparative study and also in his paper with Weidman, noted degenerative changes in the ganglion cells, increase of neuroglia and myelin sheath degeneration; Rusk found the nerve cells normal. Most observers have completely ignored these elements, confining their attention entirely to the organisms and the more prominent changes produced by them. In addition, my first case showed an infarct in the pons and a marked atrophy of the cerebellum.

The portal of entry is probably the respiratory tract in most cases. Torulae were found in the lungs of several cases (Hall, Hirsh and Mock, Pierson, Rusk and Farnell, Sheppe, Stone and Sturdivant, White, and Williams) of which Sheppe's case has the distinction of being the only one without clinical signs of cerebral involvement. Many others had pulmonary disease of another nature: pulmonary tuberculosis (von Hansemann, Lynch and Rose, Rappaport and Kaplan, Stoddard and Cutler, Versé); tuberculous bronchial lymph nodes (Hansmann); terminal pneumonia (Masse and Rooney, Pierson, Stoddard and Cutler, Smith and Crawford). Torulae were cultured from the upper respiratory tract in three cases (Evans, Rappaport and Kaplan, Türck), tonsillitis preceded the illness in three (Freeman and Weidman, Jones, Sheppe) and otitis media in three (Bettin, Stoddard and Cutler, and Wilhelmj). Alvarez described a red torula with cultural characteristics like the others, grown from a patch of reddish hair-like filaments on the tongue, which produced symptoms by tickling the uvula; there were no systemic symptoms. Berghausen cultured a torula from an ulceration of the tongue which developed following an injury; this was associated with pleurisy and X-ray evidence of consolidation of the lungs, and a palpable spleen. The patient died of inanition. Curetting from nodules in the pharynx revealed the organism to Jones, in a patient in whom all of the symptoms were local for months but later became systemic.

No treatment is known which affects the course of the disease, but Stone and Sturdivant have inhibited the growth of the organism *in vitro* by the use of gentian violet, gold sodium thiosulphate, and X-ray therapy.

SUMMARY

1. Two cases of torula infection are presented. In the first the infection was generalized but the symptoms were almost entirely cerebral. A remarkable collection of pathological changes were present in the brain: diffuse meningitis, granulomas in the meninges, marked endarteritis and proliferation of adventitial elements of the meningeal vessels, an infarct in the pons, areas of softening and focal disappearance of the granular and Purkinje cells in the cerebellum, diffuse ganglion cell changes, increase of neuroglia, nerve fiber destruction, myelin sheath damage, and encephalitis by extension in the striate body. The second case falls into the group which Freeman considers embolic phenomena.

2. Mucicarmin was found to be an excellent differential stain, not only making it easy to identify the organism by its distinctive color, but bringing out details of structure not hitherto recorded.

3. Two strains of yeast-like bodies were isolated: the one producing no pigment falls into the torula group; the one producing pigment appears to be chromotorula. The organism was non-pathogenic for guinea pigs, rats and mice.

4. The respiratory tract is probably the portal of entry in most cases. The infarct in the pons, endarteritis of numerous meningeal vessels with torula in the intima, and softenings in the cerebellum in Case 1 add weight to the theory that cystic cavities in the deep white and gray matter are embolic phenomena and dissemination is by the blood stream.

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Mixer in spinal epidural granuloma. Seven cultures made of the cerebrospinal fluid on blood agar and Rosenow's medium were negative after three days. Of twenty-three cases in which cultures were attempted, growth was obtained in seventeen; in one of these, cultures were negative in the early stages of the disease. In six no growth was obtained, in spite of the fact that the organism was seen on smear of the fluid.

Harrison's classification of the torulaceae on physiological characteristics appears to be the best to follow and is recommended by Henrici who says: "He retains the genus *Mycotorula* of Will for those forms producing rudimentary mycelia. The remaining species are grouped according to pigment production into the genera — *Rhodotorula* with red pigment, *Chromotorula* with pigment other than red, and *Torula* with no pigment. *Torula* and *Mycotorula* are further divided into groups according to their sugar fermentations."

- Group A.* No acid or gas in any sugar.
- Group B.* Slight acid in dextrose, mannose, fructose or galactose.
- Group C.* Slight acid with or without trace of gas in dextrose, mannose, fructose or galactose, and saccharose.
- Group D.* Marked acidity and gas in dextrose, fructose, or galactose, and mannose.
- Group E.* Marked acidity and gas in dextrose, mannose, fructose, galactose and saccharose.
- Group F.* Marked acidity and gas in dextrose, mannose, fructose, galactose, saccharose and raffinose.
- Group G.* Marked acidity and gas in dextrose, mannose, fructose, galactose, saccharose and maltose.
- Group H.* Marked acidity and gas in dextrose, mannose, fructose, galactose, saccharose and lactose.
- Group I.* Marked acidity and gas in dextrose, mannose, fructose, galactose, saccharose, lactose and inulin.

According to this classification the strain producing no pigment falls into *Group D* of torula. The strains producing pigment appear to be chromotorula.

Torula infection should be considered in the differential diagnosis by clinicians where symptoms of increased intracranial pressure of unknown etiology, prolonged chronic meningitis, and chronic pulmonary conditions are present. The organism should be looked for in the cerebrospinal fluid and the sputum. Likewise, pathologists should be more guarded in calling all chronic meningeal and pulmonary disease tuberculosis when the tubercle bacillus is not found.

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DESCRIPTION OF PLATES

PLATE 29

FIG. 1. Case 1. The pia-arachnoid is thickened and there are many granulomas in the Sylvian fissure.

FIG. 2. Case 2. Cystic cavities 1 to 4 mm. in diameter are seen in both lenticular nuclei.

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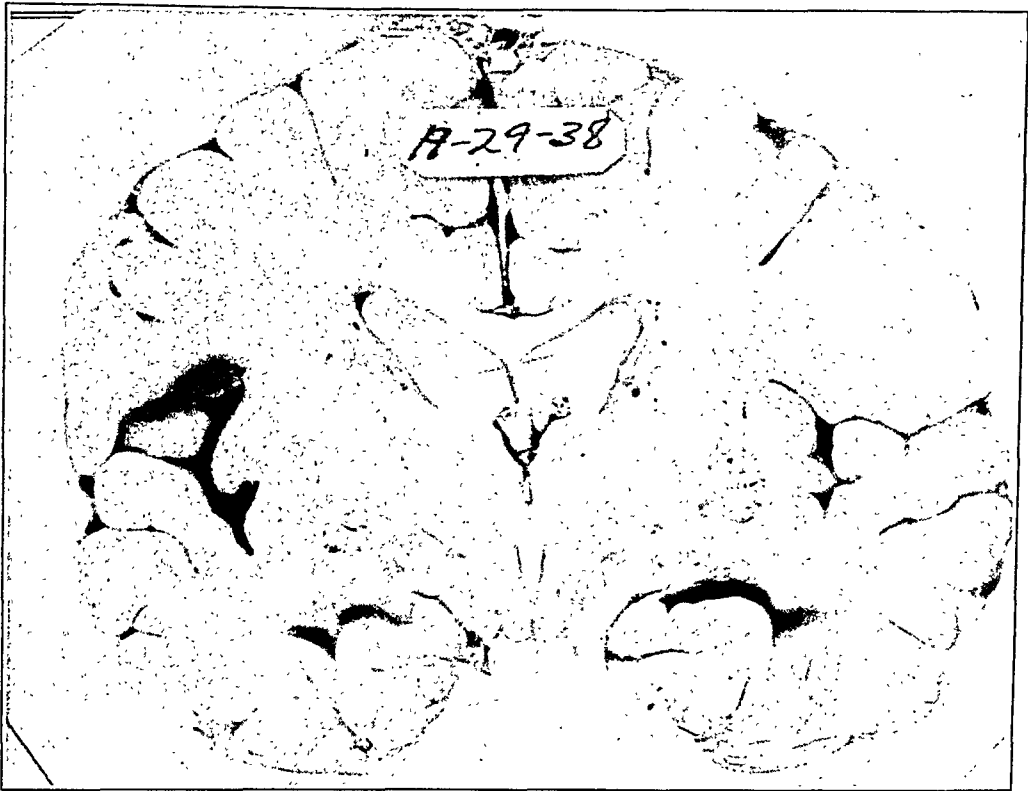
PLATE 30

FIG. 3. Case 1. An abscess containing many torulae lying in necrotic material is shown on the right, and normal adrenal on the left. The organisms vary considerably in size; some are surrounded by clear zones. Mucicarmin stain. $\times 600$.

FIG. 4. Case 1. Section through a granuloma in the meninges with numerous torulae and a few giant cells near the center surrounded by a fibroblastic reaction. Mucicarmin stain. $\times 600$.



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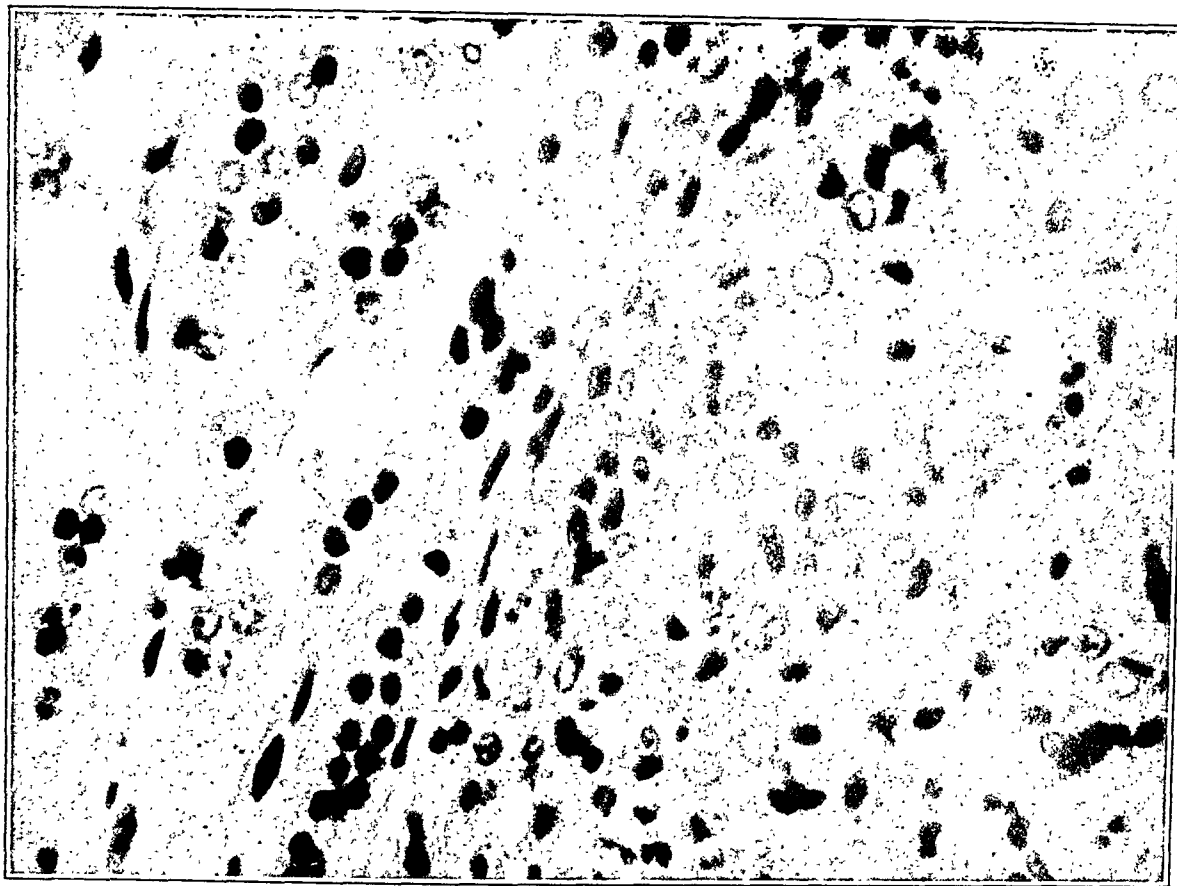
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PLATE 31

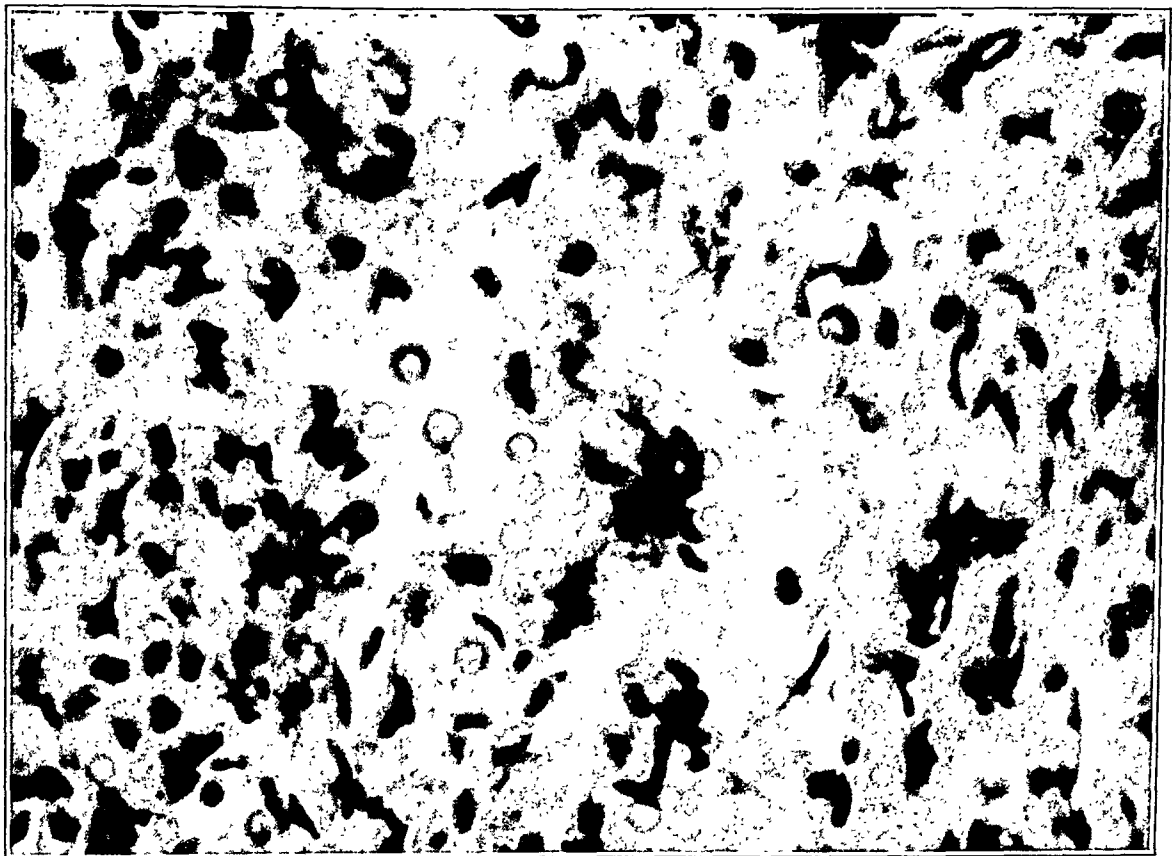
FIG. 5. Case 1. Drawing of Fig. 4 at a higher magnification. Plasma cells and cells with large pale staining nuclei predominate, and numerous lymphocytes are present. Organisms budding, with double contours, having spicules, surrounded by clear zones, and with nuclear-like material are shown. Mucicarmin stain. $\times 1000$.

FIG. 6. Case 1. Drawing of an abscess in the adrenal. Details of the structure of the organism are shown. Mucicarmin stain. $\times 1000$.

FIG. 7. Case 1. A budding torula in the adrenal. Mucicarmin stain. $\times 1000$.



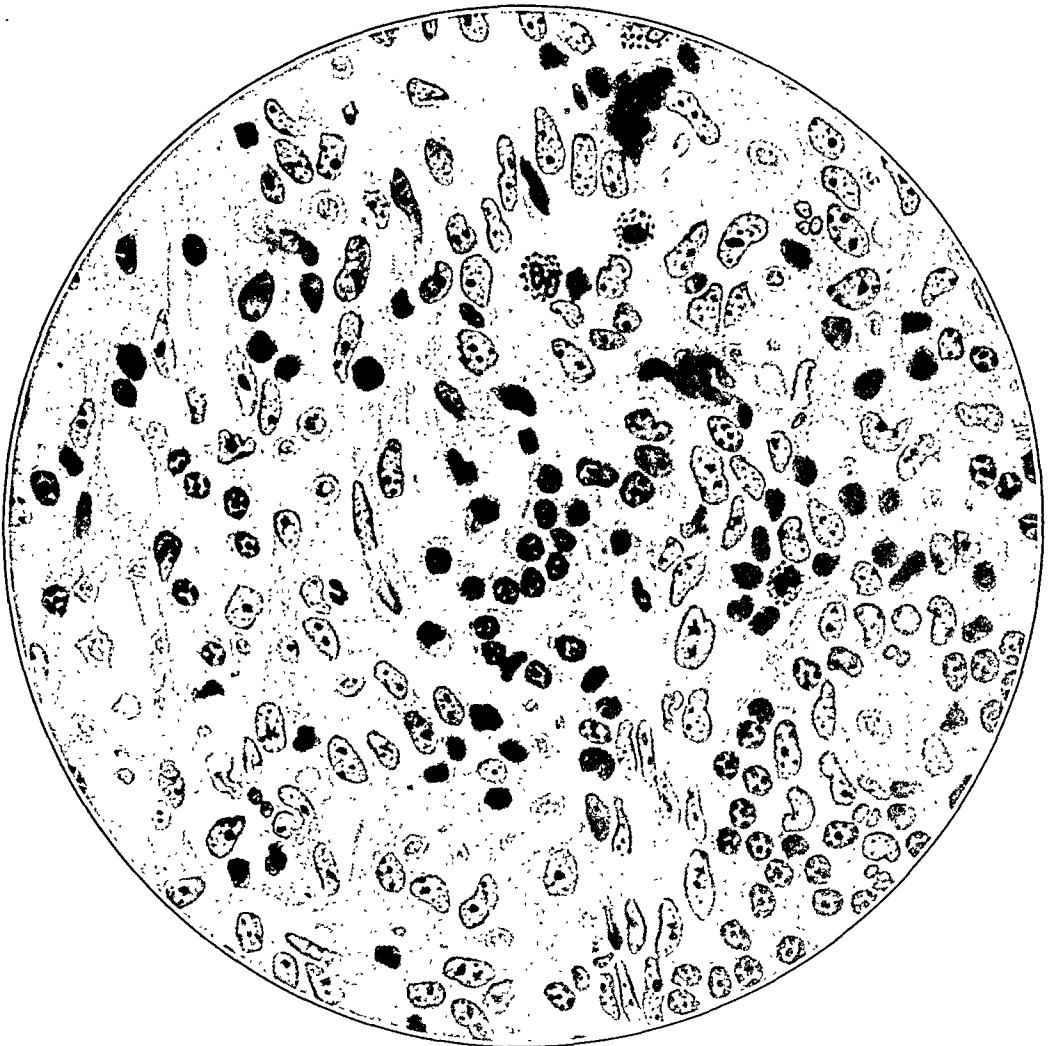
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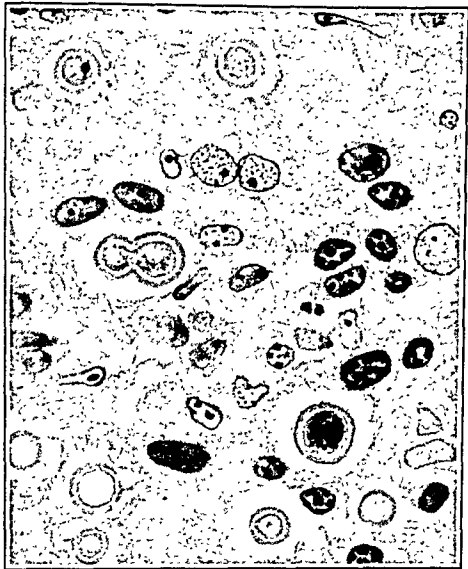
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PLATE 32

- FIG. 8. Case 1. There is an absence of the Purkinje and granular cell layers of the upper half of the folium; the lower half is almost normal. Thionin stain. $\times 60$.
- FIG. 9. Case 2. Cystic cavities in the lenticular nucleus with very little inflammatory reaction about them contain many organisms. The black angular material is debris. Hematoxylin and eosin stain. $\times 60$.



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PLATE 33

FIG. 10. Case 1. Superficial and deep colonies of the non-pigmented strain cultured from the spleen. Actual size.

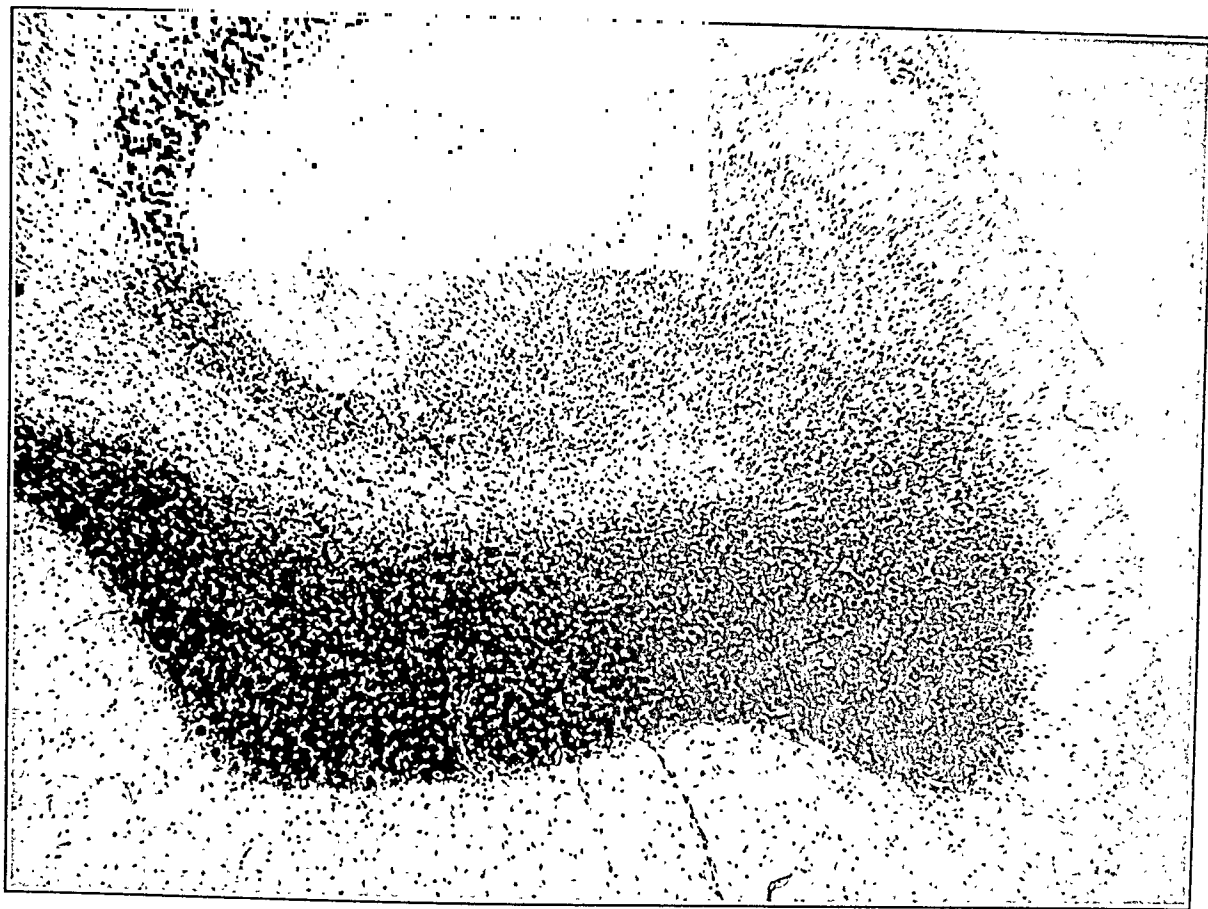
FIG. 11. Case 1. A tubercle-like nodule and blood vessels with exudate about them on the frontal pole.

FIG. 12. Case 1. Colonies of the pigmented strain of torula isolated from the lung of a guinea pig injected with cerebrospinal fluid. Actual size.

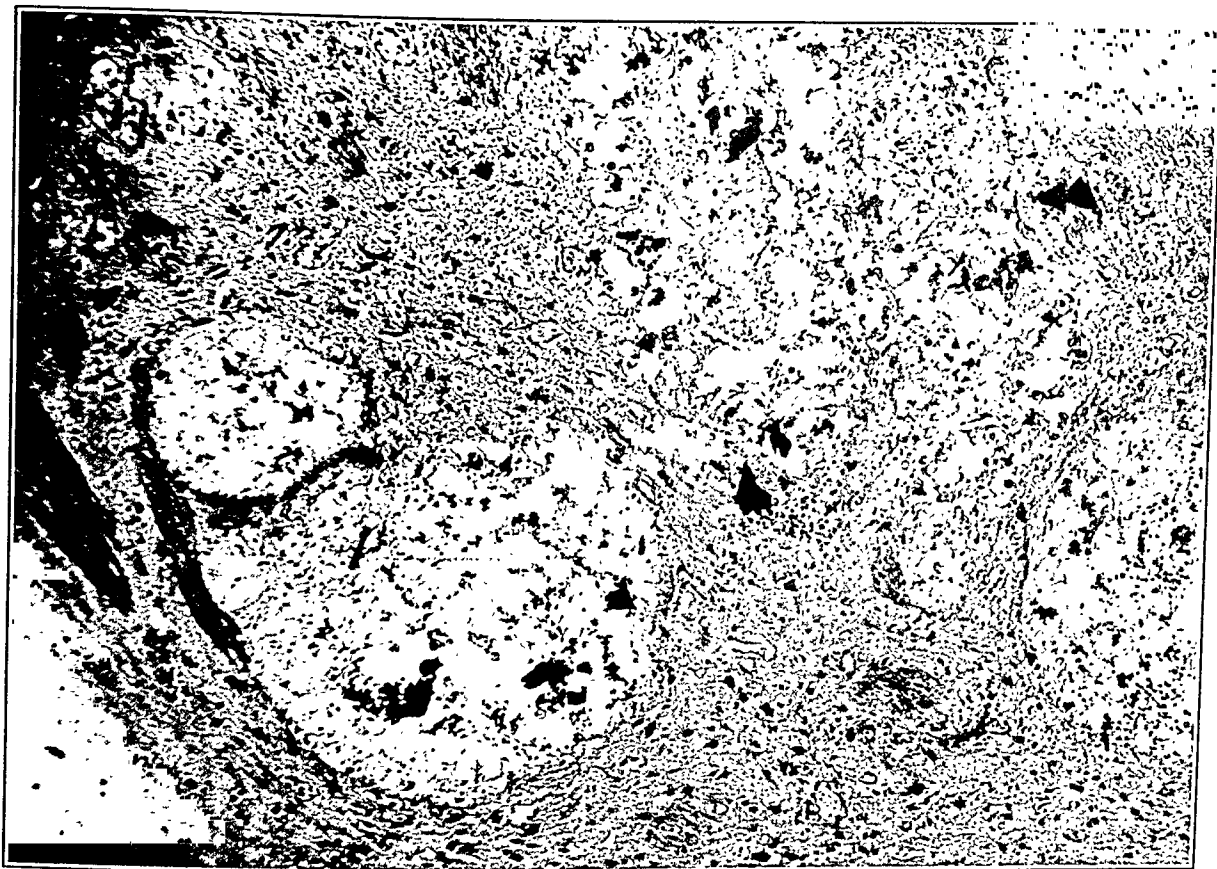
FIG. 13. Case 1. Organisms within and without a giant cell in the meninges. Mucicarmin stain. $\times 600$.

FIG. 14. Case 2. Cystic cavities near the dentate nucleus of the cerebellum. Actual size.

FIG. 15. Case 2. Yeast-like organisms with double contours in a cystic cavity in the lenticular nucleus. Hematoxylin and eosin stain. $\times 1000$.



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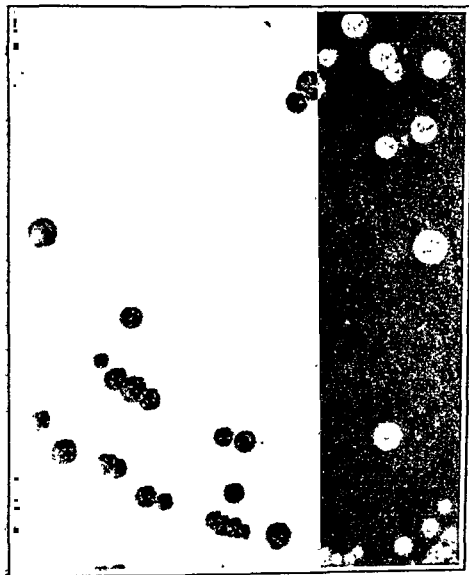




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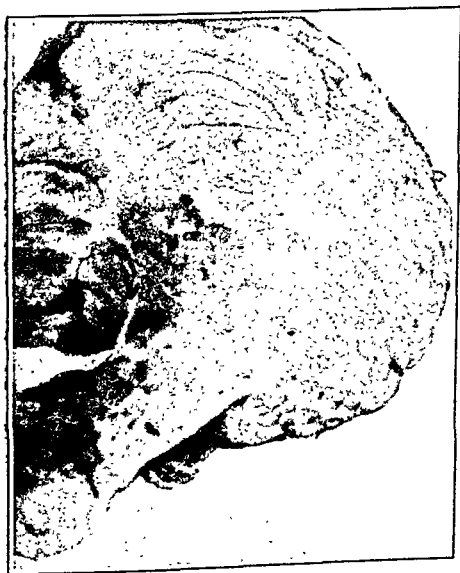
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general group of hyaline degenerations of cell protoplasm. Nesti¹⁵ was led, as the result of animal experimentation, to a similar conclusion, that is, the waxy change is merely an end stage of an albuminoid metamorphosis, granular and hyaline changes being earlier stages. Beneke's experimental work led him to the same conclusion.¹⁴

The present work was undertaken to consider whether or not the classical waxy degeneration as described by Zenker is a true pathological entity, and to study some of the factors involved in its production.

EXPERIMENTAL

Healthy, half- or full-grown rabbits were used for most of the experiments. A few white mice were used for bacterial injections and two dogs for study of the effect of high body temperatures: Except when otherwise stated, the animals were fed an ordinary mixed diet. Four main types of muscle injury were employed:

Group 1. Physical Injury: Ligation of the arterial blood supply, mechanical trauma, artificial hyperpyrexia and freezing.

Group 2. Chemical Injury: Lactic acid injections; *in vivo* injections into the arterial blood supply to the muscle, intravenous injections, or direct injections into the muscles themselves; *in vitro* dropping of muscle slices directly after removal from the body into various concentrations of lactic acid in normal saline. The effect of different nutritional states of the animals on the type of change was also studied in this group.

Group 3. Bacterial and Parasitic Injury: Injection of bacterial filtrates, living cultures or parasitic substance intravenously, intraperitoneally or intramuscularly.

Group 4. Pharmacological Injury: Injections of strychnine and insulin in fasted and normally fed animals.

Amytal by intraperitoneal injection was used to obtain complete anesthesia for all operative purposes. Injections into blood vessels were made with hypodermic needles so that the blood flow was disturbed as little as possible during the injection. Tissues were stained with Harris' hematoxylin and eosin and with Mallory's triple connective tissue stain.

Type protocols are given below.

STUDIES OF EXPERIMENTAL MUSCLE DEGENERATION *

I. FACTORS IN THE PRODUCTION OF MUSCLE DEGENERATION

D. K. FISHBACK, M.S., AND H. R. FISHBACK, M.D.

(From the Department of Pathology, Northwestern University Medical School, Chicago, Ill.)

The work under discussion deals with a type of striated muscle change known variously as Zenker's degeneration, waxy degeneration, Zenker's hyalin, Zenker's necrosis, vitreous degeneration and hyaline degeneration. Zenker,¹ in 1864, was the first to describe it thoroughly in his classical monograph, using material obtained during the great Dresden typhoid epidemic of 1859-1862.

Zenker described the change which is found most frequently in the adductors of the thigh and the recti abdomini in typhoid fever as consisting in the "conversion of the contractile substance of the primitive bundle to an entirely homogeneous, colorless, strongly wax-like refractile mass, with complete disappearance of the cross-striations and destruction of the muscle nuclei, while the sarcolemma is retained." He named it waxy ("wachsartige") degeneration. Zenker described the gross and microscopic appearance of the fibers and noted the spottiness of the change, the fragility of the fibers, the gross ruptures with hemorrhage and the microscopic separation of contractile material within the sarcolemma sheath. He believed that this change was non-inflammatory in origin, but was due to the forcing in of albuminous substances from outside the fiber because of trophic nerve disturbances from spinal cord damage.

Waxy degeneration of skeletal muscles was observed by Zenker not only in typhoid fever, but also in tetanus, cholera, scarlet fever, and possibly in typhus fever and miliary tuberculosis. Subsequent observers have reported its appearance in various infections such as trichinosis,^{2, 3} influenza and pneumonia,³⁻⁷ tetanus,^{3, 8, 9} and in diverse conditions such as anaphylaxis,^{10, 11, 12} in the neighborhood of malignancies,¹² and in epilepsy.¹³

Von Recklinghausen (cited by Beneke¹⁴) was the first to consider waxy degeneration not as an entity but merely as one type of the

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fine granules. The sarcoplasm of others seems to be alternately condensed and rarefied into discoid segments without, however, any intervening breaks in the continuity of the contractile substance. (2) More of the fibers are swollen, with loss of cross striations and prominence of longitudinal fibrils, which tend to separate from one another with a vacuolization, which in extreme cases gives the fiber the appearance of a network. In most of the fibers the sarcolemma is intact, but occasional ruptures are seen. Numerous erythrocytes are seen in the interstitial tissue.

3. *Hyperpyrexia*: Examinations were made of the recti abdomini and diaphragmatic muscles of two dogs whose body temperature had been raised to 110° F for one-half hour by means of a high-frequency machine. The dogs became ill and died in a few hours after the treatment.*

No abnormal changes of the muscles were noted grossly or microscopically.

4. *Freezing*: The gastrocnemii of several rabbits were frozen quickly *in situ* with a slush of carbon dioxide snow and ethyl chloride. 4 to 72 hours later the muscles were removed and placed in warm formalin.

Grossly they were moderately swollen, and surrounded by a pink to yellow fibrin exudate. Microscopically there is moderate patchy edema. The muscle fibers show all grades of degenerative change from simple swelling to complete disruption, as in those injured by mechanical trauma, although less extensive and usually showing fewer of the severely injured fibers. There is marked hyperemia but no thrombosis of the vascular channels. Exudative cells are rather few, and the majority are mononuclear phagocytes with a smaller number of polymorphonuclear leucocytes.

Group 2. Chemical Trauma

1. *In Vitro Experiment*: Animal killed by sharp blow on back of head. Large portions of lumbar muscle were excised and cut rapidly into blocks about 5 mm. in thickness, which were immediately dropped into lactic acid solutions of various concentrations in saline at 37° C for varying lengths of time. The lactic acid concentrations varied from 0.001 per cent (0.00011N) to 1.0 per cent (0.11N),

* We desire to thank Dr. Bernard Mortimer for the opportunity of studying the muscles of these animals.

Group 1. Physical Trauma

1. *Ligation Alone:* Femoral artery of one side of amyralized rabbit ligated with aseptic technique. Animal killed by blow on back of head 18 hours later. Gastrocnemii removed at once and placed in 10 per cent formalin at 37.5° C.

Control muscles showed no abnormal change grossly or microscopically. Ischemic muscles showed grossly merely marked pallor. Microscopically there is slight change. Some fibers are moderately swollen, stain feebly with eosin and show scattered fine granules in the cytoplasm.

2. *Contusion: (a. With Ligation:)* Femoral artery on one side tied off. Gastrocnemius on that side injured by sharp blows with a wooden board, and hamstring muscles on same side injured by pinching with heavy forceps. Animal killed by sharp blow on back of head from 2 to 5 hours after ligation.

Both muscles showed grossly pallor, swelling and mottling with pinkish red and grayish white streaks. The tissues were very friable and opaque. Histologically there is considerable change, from swelling of the fibers with loss of cross striations to complete lumpy disruption of the contractile substance. A few fibers show vacuolization of the sarcoplasm. In many of the extremely degenerated fibers there are breaks in the sarcolemma sheath. Normal and completely disrupted fibers may lie side by side. No hemorrhage is evident and the vessels appear to be drained of their blood. In the gastrocnemius of one rabbit, in which both the femoral artery and vein were ligated, extensive vacuolization of the fiber substance was observed.

(b. *With Circulation Intact:*) Gastrocnemius and hamstring muscles of the other leg, with circulation intact, injured as above, and lumbar muscle bruised with wooden board.

The muscles were dark red in color from hemorrhage which stained the ruptured fibers and were tensely swollen and easily torn with handling. Microscopic examination reveals marked and extensive damage. Muscle bundles are broken up and the fibers show considerable disruption. There are two main types of change: (1) Some of the fibers are smooth and hyaline, staining darker red than normally with eosin, and their cross striations are either indistinct or absent. In some of these the cytoplasm is clouded with

(c) Intramuscular injection of 2 cc. of warm 2 per cent acid into the lumbar muscles of a lipemic animal whose femoral artery had also been injected. Animal sacrificed $1\frac{1}{2}$ hours after lumbar injection, and immediately after the arterial injection.

Lumbar muscles showed at site of injection a slight hemorrhage with diffuse surrounding edema. The whole area measured about 4 cm. in diameter. The tissue was opaque, grayish white in color and friable. There is interstitial edema throughout the sections. Many fibers show swelling and loss of cross striations. Longitudinal fibrils are often prominent. The typical change is a vacuolization, small to large, with separation of fiber substance.

Leg muscles on injected side are similar to the above-described muscles of non-lipemic animals.

Group 3. Bacterial and Parasitic Trauma

Materials: A highly virulent strain of *B. mucosus capsulatus* was obtained at autopsy from the lung of a man who had died of pneumonia. The stock culture was kept on blood agar, transfers being made as needed to dextrose agar slants containing a small quantity of broth.

The strain of *Streptococcus hemolyticus* used was obtained from the lungs of a rabbit which had died of empyema.

The strain of *Streptococcus viridans* was obtained from the infected tooth of a man.

In all instances the cultures used for injection were 24 hour growths in nutrient broth.

The *Ascaris lumbricoides* were obtained from sheep intestines at the slaughter house. The worms were washed thoroughly in water, dried in a current of slightly warmed air, ground up finely and passed through a fine screen so that an impalpable powder was obtained. Injections were made of this powder suspended in physiological salt solution.

The diphtheria toxin used had an M. L. D. of 0.004 cc.

1. *Intraperitoneal Injections Into Mice of 1 or 2 cc. of Living Cultures of Streptococcus hemolyticus and of B. mucosus capsulatus:* All animals became extremely ill very shortly. The streptococcus-inoculated mice were killed with ether in about 10 hours, the others died within 4 hours. Muscles of the entire body were fixed at once in 10 per cent formalin.

and the time intervals from 30 seconds to 1 hour. Each of the forty-five specimens thus obtained was stirred in a large volume of warm 10 per cent formalin immediately on removal from the acid.

No gross muscle change was discernible. Microscopically the muscles are without change except at the ends of the fibers, which are swollen and knob-like and show loss of cross and longitudinal striations. Even in the weakest acid solutions, as well as in the strongest, the same changes are seen.

2. *In Vivo Experiments:* (a) Warm lactic acid (2 per cent in saline) injected into femoral artery over a period of from 3 minutes to 1 hour in normally fed animals, into animals fasted 4 days, and into one animal in which a lipemia had been produced by the feeding of 130 cc. of cream by stomach tube 4 hours before the beginning of the injections. All animals were sacrificed immediately after, or within 25 minutes of cessation of injection, vessels ligated, and the muscles removed at once and placed in warm formalin.

Gastrocnemii injected with 5 cc. of 2 per cent lactic acid over a period of 3 to 5 minutes showed no change grossly or microscopically.

Gastrocnemii injected with from 25 to 50 cc. of 2 per cent lactic acid over periods ranging from 5 minutes to 1 hour showed occasional, irregular, zig-zag streaks of grayish white crosswise in the somewhat edematous muscle. The tissue was pallid and translucent, but not more friable than normal. Histologically various types of muscle degeneration are seen. Some fibers are swollen, opaque and have a shining hyaline appearance, staining more deeply with eosin than normal fibers. These fibers have intact nuclei and sarcolemma sheaths. Other swollen fibers are pale staining, with indistinct cross striations and conspicuous longitudinal fibrils. Their sarcoplasm tends to be alternately rarefied and condensed, although no actual spaces are seen. In some fibers there is exaggeration of cross striations, which look as though they were set in a colorless matrix. Occasionally there are small areas in which the fibers appear to have normal morphology.

(b) 135 cc. of warm 2 per cent acid injected into femoral vein over a period of 1 hour. Dyspnea noted during injection, especially during the times when the acid was actually flowing into the vein, and less marked during the short rest periods.

Muscles of the legs, back and diaphragm showed no abnormal change.

(b) *Ascaris Substance Injected Intramuscularly*: Animal killed 24 hours later.

All injected muscles fixed in formalin and Zenker's fluid.

At the injected site in the muscles was a central yellowish gray area which was moist and friable. This was surrounded by a narrow, pinkish red zone, outside of which the muscle appeared to be unchanged. Affected muscles showed occasional grayish white spots a few millimeters in diameter.

Microscopically the inoculated site is outlined by a zone of polymorphonuclear leucocytes, outside of which the fibers are separated by edema fluid. There is dimming of the cross striations and moderate swelling of the fibers. Some of them show beginning disruption of the cytoplasm into irregular masses.

In the center of the inoculated area there is muscle destruction and pus cell infiltration. Bordering this central necrotic area there is an irregular zone in which the muscle fibers show varying degrees of degeneration. Many fibers are swollen and most of them are hyaline with indistinct or absent cross and longitudinal striations. In many the longitudinal striations become more distinct and occasionally tend to pull apart slightly. Some take a pale eosin stain, while a few become plump, opaque, take a deep eosin stain and have a glistening appearance. In many of these fibers there is disruption of the cytoplasm into irregular masses which separate, leaving clear spaces within the sarcolemma sheath. There is partial absorption of these degenerated fibers.

An isolated mass which resembled lymphoid tissue was found in the adductor muscles of a hind leg of one rabbit. It was removed to warm formalin. The mass measured 3 by 2 by 2 cm. and was attached only along its border next to the femur. It was surrounded by a reddish yellow zone of exudate. On section the tissue was pinkish gray in color, soft and succulent, and very friable. Microscopic examination reveals an isolated muscle mass surrounded almost entirely by a zone of young granulation tissue. The interior of the mass is composed of degenerated muscle fibers. These are swollen, entirely homogeneous and glistening in appearance, and take a strong eosin stain. Some of these masses show a breaking down into granular clumps with disruption of the sarcolemma sheath. The fibers are in general rather widely separated and numerous pus cells and debris are present. At one end regeneration of

Grossly there was marked, acute peritoneal inflammation with fibrinous or fibrinopurulent exudate, but no abnormal skeletal muscle changes were observed.

Microscopically there is little change. The diaphragmatic and heart muscles show the finely granular change characteristic of cloudy swelling. The only other change is seen in the fibers surrounding a small abscess in the abdominal wall of one animal at the point where the needle entered. The altered fibers in this zone are completely hyalinized and glistening and take a deep eosin stain.

2. *Intravenous Injections Into Rabbits of Living Cultures of Streptococcus hemolyticus and of Streptococcus viridans:* Rabbit X₃ 1 cc. *Streptococcus hemolyticus* into ear vein October 23, 1 cc. on October 30. Rectal temperature at time of first injection 104.5° F; 5 hours later 104.4°; 24 hours later 105.5°. Temperature 30 minutes after second injection 105.5° F. Rabbit died 1½ hours later. Tissues fixed at once in Zenker's fluid.

No gross changes were observed and microscopic examination shows only slight cloudy swelling, most marked in the heart.

Rabbit X₄. 1 cc. of *Streptococcus viridans* into ear vein October 23, and 10 cc. October 30. Animal ill. 20 cc. intracardiac on November 7. Animal died at once. Muscles fixed in formalin and Zenker's fluid. Rectal temperature at time of first injection 103.2° F; 5 hours later 106.1°; 10 hours later 104.8°; 22 hours later 103.3°; 27 hours later 103.5°. Rectal temperature at time of second injection 104.8° F; 24 hours later 104.9° F.

Autopsy revealed hemopericardium and fibrinopurulent pericarditis with petechial subpericardial hemorrhages. No gross skeletal muscle changes were observed.

The lumbar muscles show no microscopic changes. The diaphragmatic and leg muscles show occasional areas in which the fibers are swollen within the sarcolemma sheath. Cross striations are indistinct or absent and fine granules may be seen throughout many of the fibers. Here and there are groups of fifteen to forty muscle nuclei gathered within one sarcolemma sheath. A few mononuclear phagocytic cells are present in the interstitium.

3. (a) *Intramuscular Injections Into Rabbits of Living Cultures of Streptococcus hemolyticus, Streptococcus viridans, and B. mucosus capsulatus:* Rectal temperatures showed no significant alteration. Animals sacrificed in 40 to 50 hours.

(b) *2 mg. per Kilo Injected Into a Rabbit Which Had Been Fasted for 4 Days:* Convulsions beginning 1 hour later and spastic paralysis of the hind legs. Second dose of 1 mg. per kilo 2 hours after the first resulted in a severe convulsion and death 20 minutes later.

Sections of muscles of back, legs, and the intercostal and abdominal muscles show no change.

2. *Insulin, 10 Units, Injected in Upper Back Muscles of a Rabbit Which Had Received No Food for 43 Hours:* 3 hours later, after growing restlessness and hyperirritability, a series of mild convulsions lasting for about 10 minutes. 8 more units of insulin injected 1 hour later into upper back muscles of other side. 2 hours later strong convulsions, legs spastic. Convulsions at intervals. Animal growing weaker and less responsive. Killed by blow on back of head 7 hours after first injection.

No significant alterations observed in muscles of the legs, diaphragm, abdomen or the intercostals.

DISCUSSION

As might have been expected, the early gross evidences of muscular trauma caused by pinching and striking of the muscle with intact circulation are swelling and hemorrhage. The friability results partly from the traumatic separation of fibers, as well as from the degeneration which occurs. Microscopically this degeneration is found to comprise various types of fiber damage. The beginning change is simple swelling with loss of cross striations and fading of the eosin-staining property. In many of these fibers the longitudinal fibrils stand out distinctly. In such fibers the further progress of degeneration is marked most characteristically by vacuolization. When this change is extreme the swollen fiber appears to be made up of a filmy network of bubbles. According to Wagener¹⁶ this vacuolar change is a purely degenerative process which results in complete destruction of the fiber. Other fibers, usually scattered singly here and there among intact fibers or among fibers showing other types of injury, are strikingly marked out by their bright red color, opaque appearance and completely hyaline structure. They have a shiny look. Rarely the swollen fibers have the appearance of cloudy swelling, with their cross striations partially or completely obscured by the appearance of small glistening granules which

muscle is seen, with myriads of young muscle sprouts present. A regional lymph node shows marked diffuse lymphoid hyperplasia.

4. *Diphtheria Toxin Injections*: 2 cc. of undiluted toxin intramuscularly; 30 cc. of toxin diluted 1:3 with saline intra-arterially with circulation intact; and perfusion of the leg muscles with 1:3 toxin; all within 1 hour. Animals sacrificed at once and muscle fixed in formalin.

There is hemorrhage and edema in the directly injected muscles. The others show only pallor.

Microscopically the directly injected muscles show moderate edema of interstitial tissues with moderate swelling of fibers, indistinct cross striations and prominent longitudinal fibrils. Nuclei and sarcolemma sheaths are intact. The perfused muscles show no abnormal change.

Group 4. Pharmacological Trauma

1. *Strychnine, by Subcutaneous Injection*: (a) 4 mg. per Kilo Into a Normally Fed Rabbit: Gave hyperirritability, rigidity, prostration, with muscle twitchings and convulsions beginning in 3 minutes, and death 15 minutes after injection. Muscles of legs, back, neck and diaphragm removed at once and placed in formalin.

Grossly there was no abnormal change, but microscopic examination revealed some muscle damage. In the diaphragm occasional fibers show granular degeneration of the cytoplasm and loss of cross striations. Some of these show further degeneration into broken granular masses inside the sarcolemma sheaths. Many muscle nuclei are seen, often irregularly distributed throughout the granular mass and surrounded by small cytoplasmic rims. Occasionally these degenerated fibers are completely fragmented and in one place where a few such fibers are broken across there is a small effusion of blood mingled with muscle fiber fragments and numerous muscle cell nuclei.

Sections of the leg and back muscles show swelling of the fibers at their points of attachment to the fascia, where there is loss of cross striation with prominence of longitudinal fibrils and separation of the longitudinal fibrils from each other. Some fibers show a diffuse opacity, due to clouding with fine granules. The sarcolemma sheath is intact. The remainder of the fibers have normal morphology.

gross or microscopic evidence of injury. The rabbits which were checked for body temperature following bacterial injections showed no connection between temperature observed and muscle degeneration resulting. As to the effect of temperatures considerably above those developed by the animal body, Volkmann² made observations on muscle degeneration caused by burning, cauterizing, and injecting hot water, and found not waxy degeneration but complete necrosis of the affected muscle, with healing by scar formation.

In addition to the actual physical damage of the traumatized muscle there is set up in the injured area an alteration of metabolism which may lead to accumulation of damaging products. It is logical to assume that one product accumulating rapidly in such a situation is lactic acid. In 1909 Wells¹⁹ first advanced the theory that waxy degeneration of skeletal muscles is caused by this collection of lactic acid in the muscle. He found a striking homogeneity of the swollen fibers resembling that of Zenker's degeneration, when he placed muscle tissue into solutions of lactic acid in saline. Even as dilute a solution as 1:64N lactic acid gave, in an hour or two at 37° C, distinct swelling of the ends of the fibers, loss of transverse and obscuration of longitudinal striations. Since sodium lactate did not produce this change and hydrochloric acid did, Wells concluded that it was the hydrogen ion which was the important factor. Wells gives the figures of Fletcher and Hopkins²² which show that enough lactic acid to produce the change can accumulate in living muscle. Although their methods have since been shown to yield too high figures, more recent workers²³ have obtained even higher values for rat muscles exercised to complete fatigue.

In our experiments with lactic acid *in vitro* no muscle change was observed. The cut ends of the fibers were knob-like and hyalinized in specimens at all the lactic acid concentrations studied, but since similar changes were observed in the controls which were placed in saline solution, this change was interpreted as being due to rupture of the fiber sheath in cutting the sections, as reported by Thoma in 1909.

Quite different results are seen in the living animal injected with lactic acid, either into the blood supply of a part of the body or directly into the muscle. The most extensive changes are noted on the direct injection of the injurious agent into the muscle. No detectable differences are observed in muscles of the animal with

sometimes have a slightly yellowish tinge. This granular appearance may, according to Nesti,¹⁵ presage the final conversion of the fiber into the waxy form of degeneration.

In all of these described forms of degeneration the sarcolemma sheath may be intact, with the contained muscle nuclei uninjured. Many sheaths, however, have been ruptured from the force of the trauma, and in these the muscle nuclei may be found escaped in the outpoured cytoplasmic mass in varying stages of degeneration, or at times completely degenerated.

As to the underlying factors leading to muscle degeneration in this type of trauma, the actual traumatic agent must be considered first. It might be expected that trauma to the muscles would be more effective if their circulation were impaired, since this would facilitate accumulation of toxic metabolic products. In the muscles injured by striking and pinching after ligation of the arterial blood supply extensive degeneration of the fibers is evident. According to Thoma¹⁷ this decreased resistance of fibers is due to lack of nourishment, the combination of decreased food and mechanical injury producing the degeneration which, according to him, either injury alone fails to effect. In our experiments, however, about as much damage was observed with intact circulation as in the ligated muscles. Also, since degeneration does occur without hemorrhage, the factor of blood effusion cannot be considered a basic factor in the causation of the degenerative change.

The damage produced by ligation alone is of slight extent and compares with the change seen in parenchymatous organs with cloudy swelling. This lack of significant change with simple ischemia is affirmed by Thoma¹⁸ and Wells.¹⁹ Contrary opinions, on the other hand, are expressed by Volkmann² and Siegmund.²⁰ Voelcker (cited by Krogus²¹) was the author of a theory that the basic cause of congenital torticollis is pressure of the shoulder of the infant *in utero* upon the upper end of the sternocleidomastoid muscle where the artery enters, with resultant ischemic degeneration of that muscle.

The effect of increased body temperature on the incidence of muscle degeneration has been considered by Fahraeus (cited by Stenström⁶), and Ghedini and Fedeli (cited by Wells⁷), who are agreed that the muscle is unaffected by fever. In our dogs with an extremely high body temperature the muscles studied showed no

added to the injurious effect of the bacteria. The extent of the muscle injury which can result from such a combination of factors is shown in one rabbit, in an isolated muscle mass resulting from a spontaneous infection. Here the muscle degeneration was extreme. A similar type of muscle mass was found by Loeper and Lemaire²⁶ in the cervical region of a man.

Closely related to the type of injury produced by direct bacterial injections is that resulting from injection of ascaris substance into the muscle. The injury with this agent may have resulted directly from the parasitic toxins.^{27, 28}

Sudden violent contraction of muscle was shown to give varying results as regards degenerative changes. In the normally fed rabbit administration of strychnine resulted in definite granular degeneration of muscle fibers and even rupture, with slight hemorrhage. In contradistinction, in fasting animals injected with strychnine and with insulin, although the convulsions were just as violent the muscles appeared unchanged. Zenker had already noted that waxy degeneration often did not occur in those muscles most involved in tetanic contractions, while appearing extensively in other muscle groups. Our findings corroborate this observation and support the view that violent muscle contraction *per se* does not constantly produce this change. Borst³ believed that it was rupture of fibers from forced contractions that led to their degeneration, while according to Stemmler¹³ rupture occurs only in those fibers which have been previously injured, as, in his experiments, by tetanus toxin.

CONCLUSIONS

The types of muscle injury resulting from different injurious agents used varied from the slightest clouding of the cytoplasm to complete necrosis of the fiber substance. The earliest change is that which was described as being similar to cloudy swelling in parenchymatous organs. By this analogy we may presume further that such fibers might recover from their damaged state without either regenerative or other reparative process. A favorable ending might be anticipated also for the milder forms of true granular degeneration (Virchow²⁹ and Zenker¹) whether this is of the albuminous or fatty type. With greater fatty change, however, according to Zenker, the cell is irreparably damaged. The albuminous and

an artificial lipemia. The lipemia was created before the injection of the acid to determine whether a difference in the amount of available fat in the circulation might influence the extent of muscle degeneration, since Rumpf and Schumm (cited by Wells²⁴) found an increase of fat amounting to about fifteen times the normal amount, in muscles showing "reaction of degeneration."

As with lactic acid, so with bacterial cultures, direct injection into the muscle causes marked degeneration. It is indicated that the effect is obtained largely by the direct action of the bacteria or their products upon the muscle fibers, for when much larger amounts of the same cultures are injected intravenously little or no degenerative muscle change is evident. The same negative results are found with acute infection of the peritoneum by virulent organisms. Since the peritoneum offers a relatively huge surface from which absorption is rapid, and since septicemia is a probable accompaniment of the peritonitis, toxins must have been present in the circulation in large amounts in these cases without, however, causing serious muscle damage.

Our findings, therefore, confirm the opinion of Beneke and Steinschneider¹¹ that the muscle damage is produced by direct injury to the contractile substance. These workers investigated the effect of direct bacterial injury to the muscle. Thoma, however, ascribed to bacteria an indirect rôle — that of disturbing the nutrition of the muscle and so rendering it susceptible to physical trauma. MacCallum²⁵ believed that the living bacteria which he found in degenerated muscle areas were secondary invaders after primary toxic injury.

Diphtheria toxin injected in large amounts, with or without active circulation, gave no change. Direct injection of toxin into the muscle produced only interstitial edema, which may be attributed to the volume of fluid injected.

Anaphylaxis has been suggested as a cause of waxy degeneration of muscle by Beneke and Steinschneider in 1912, and by Wells in the same year. In one of our rabbits (X₄) anaphylaxis must be considered the probable cause of death, and in this animal slight degenerative changes were found in the muscles of the legs and diaphragm.

It is probable that in local infections of muscle the disturbances of circulation from stasis and from thrombosis of blood vessels is

factor, perhaps of enzyme nature, is active in the degenerative transformation of muscle fibers which are already injured by some non-specific agent.

It is evident from the study of these experimental muscle lesions that there is a series of degenerative changes as described above. The stage of hyaline, or so-called waxy, degeneration is but one phase of a progressive process. There is no more reason to use the name descriptive of one stage than of any other stage in naming the entire process.

Since the degeneration is often widespread and affects the contractile units of the muscle, we would suggest the name "acute molecular degeneration of striated muscle" as being more definitive and descriptive of the entire process.

SUMMARY

1. Degeneration of skeletal muscle has been produced by different types of trauma.
2. The types of trauma used were physical, chemical, bacterial and parasitic, and pharmacological.
3. The stages of muscle degeneration produced were:
 - (a) Slight granular clouding with swelling, and dimming of cross striations.
 - (b) Edema of fibers with prominent longitudinal fibrils.
 - (c) Vacuolization.
 - (d) True granular degeneration (a) albuminous or (b) fatty.
 - (e) Waxy degeneration, with further (a) lumpy disruption, or (b) granular disruption.
4. The name "acute molecular degeneration of striated muscle" is suggested as a better descriptive and more inclusive term than "waxy degeneration."

simple hyaline forms of degeneration progress further, according to Nesti,¹⁵ into the waxy form. This waxy form is generally agreed to be completely homogeneous and of altered protein composition. The presence of fat in this waxy material has not been definitely established although, according to Forbus,³⁰ fat sometimes appears in the vacuoles resulting from the dissolution of waxy material.

Further degeneration of the waxy fiber is marked by its disruption into irregular masses separated by clear spaces (Zenker's "Schollige Zerklüftung") or by its breaking up into coarse granular particles.

Another form of degenerative change is seen in the edema of the muscle fiber accompanied by dimming of cross striations and marked prominence of longitudinal fibrils, which results from mechanical and chemical trauma. This degenerates further with the formation of vacuoles which increase in size until they occupy practically the whole space of the fiber, giving it the appearance of a network.

Muscle nuclei are present in most of the fibers which are injured by mechanical trauma, but tend in general to be absent in those fibers injured by chemical and bacterial means.

Severe mechanical trauma frequently causes rupture of the sarcolemma, although marked degeneration is frequently seen in fibers with intact sarcolemma sheath. In other types of injury the sarcolemma sheath generally remains intact until extensive destruction of fiber occurs, although it is often unbroken, even with almost complete disappearance of the contained protoplasm.

In all the types of trauma studied, fibers with normal staining reactions and with intact striations are seen lying next to fibers which show varying degrees of degeneration.

It seems questionable that the noxa alone is the direct cause of the degenerative muscle changes. Since so many widely varied agents may produce similar pictures, it is likely that the trauma sets in action some principle already present or called into being in the muscle itself. This may be the altered reaction of the muscle, due to lactic acid accumulation, although the highest value of lactic acid found in a series of injured muscles was only 103 mg. per cent.³¹ This is no higher than may be found in muscle fatigue²³ from which recovery is prompt, or within six minutes postmortem,²² which is less time than elapses before the average normal muscle specimen is fixed for histological examination. It is likely that some other

DESCRIPTION OF PLATE

PLATE 34

FIG. 1. Vacuolar change of muscle fibers. $\times 325$.

FIG. 2. Waxy change. $\times 325$.

FIG. 3. Lumpy disruption of degenerated fibers. $\times 150$.

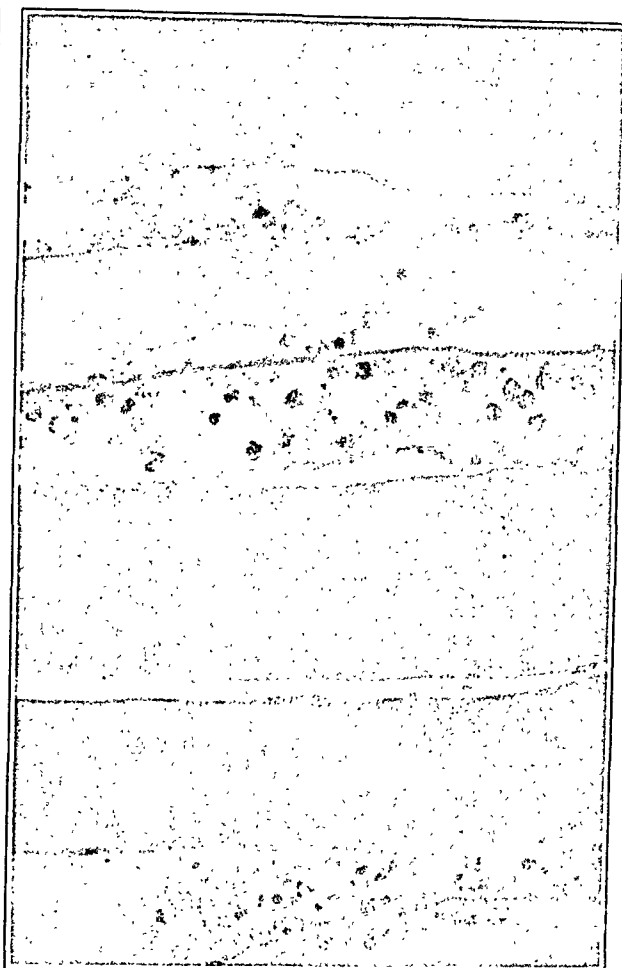
FIG. 4. Profoundly traumatized area of muscle, with marked edema and ruptured fibers showing various types of degenerative change. $\times 150$.

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I



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It also seemed worth while to report morphological studies of the injured muscles during the repair so that a more complete picture might be had of this standard muscle injury.

GROSS APPEARANCE OF INJURED MUSCLE

At 4 hours after injury the muscle is swollen to about double its ordinary size. There is edema of the subcutaneous tissues and of the injured muscles. The color is slightly cyanotic, as from blood stasis. Here and there are small, irregular, transverse, opaque, grayish white bars dotted in the muscle tissue. The muscle substance is somewhat more friable than normal.

Within 1 to 3 days the muscle loses its bluish red color and has a distinct salmon color, with contained areas of opaque grayish white tint. These areas are quite large, usually making up the greater part of the muscle, are irregular in shape and shade off at the margins into the more normal appearing muscle structure. The cut surface is not moist and glistening as in normal muscle, but appears dull or opaque and somewhat dry. The tissue is firm and quite friable.

The 5 to 10 day periods show an increasing tendency to easy bleeding of minute vessels in the area immediately surrounding the muscle, associated with apparent organization of a fibrinous exudate between the muscle and its fascia. The muscle size is decreased somewhat from the early stage of acute swelling. On section at 10 days there are small scattered areas of glistening moist tissue with the pinkish red color of normal muscle. The degenerated areas of opaque, grayish white, friable tissue show at this time a narrow, bright red to yellow-red marginal zone.

With longer periods of repair the muscle gradually diminishes in size but is still larger and more tense than normal at 28 days. The exudate between the muscle and fascia becomes firmly organized by 16 days. The proportion of more normal appearing muscle becomes much greater with disappearance of the degenerated areas, which have not entirely disappeared, however, by the end of the observation period. At their margins there is an advancing zone which fades from red to reddish gray in the late period. This zone is somewhat translucent and resists tearing. The amount of such new-forming connective tissue is never large proportionately, even in the latest period.

STUDIES OF EXPERIMENTAL MUSCLE DEGENERATION *

II. STANDARD METHOD OF CAUSATION OF DEGENERATION, AND REPAIR OF THE INJURED MUSCLE

D. K. FISHBACK, M.S., AND H. R. FISHBACK, M.D.

(From the Department of Pathology, Northwestern University Medical School, Chicago, Ill.)

In a previous paper ¹ we have called attention to the experimental production of striated muscle degeneration in which hyaline changes of the muscle fibers constitute one stage of a progressive degenerative process. We have suggested the name "acute molecular degeneration" for this form of muscle change. This change was shown to be produced by a wide variety of traumatic agents. There is considerable difference among such agents, however, as to the extent and severity of the muscle lesions. They differ especially in ease of control of the experimental lesions produced.

After many trials with various methods one was finally found which met our requirements. The method of injury consisted in contusing the muscle while the animal (rabbit) was under light ether anesthesia. With the leg extended on a well padded wooden block the gastrocnemius muscle was struck a number of scattered light blows with a light, rubber-covered iron rod. After examining several muscles injured by this means, the operator could estimate fairly accurately at the time of injury the grade of muscle damage caused.

The use of this method was of advantage for several reasons. As stated, the amount of damage to the muscle was controllable. The animal was left in good general condition, so that the injured muscle might be studied at any stage of its progress. The skin was unbroken, with danger of infection thus minimized. No toxic or chemical agent was introduced which might interfere with subsequent chemical study of metabolic processes in the injured muscle.

With this standard method of causing a characteristic and reproducible degeneration of muscle a series of chemical studies was undertaken in the acute stage and during the progress of repair.²

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hain, Korotneff, Stemmler, Morpurgo, and Schütz (cited by Craciun³) associated them with physiological repair.

Within 24 hours after injury there is added to the previous picture a moderate diffuse infiltration by polymorphonuclear leucocytes and lymphocytes. The muscle fibers show extensive loss of cross striations and hyalinization of the cytoplasm. There is considerable proliferation of sarcolemma nuclei at the points where the sheath is ruptured, and some very early growth of muscle cells. In some places where there is marked damage to the sheath these proliferating muscle cells are turned directly at right angles to the fiber axis. Thoma⁴ found muscle fiber proliferation in mechanically injured frog tongue muscle within 60 hours after injury.

At 48 to 72 hours after injury there is evident an increasing degree of damage. There is marked hyalinization of muscle fibers with disappearance of most of the muscle nuclei. Some hyaline fibers are already breaking up into lumpy masses or into disc-like segments. Accompanying the diminishing edema there are fewer fibers showing wide separation of longitudinal fibrils with vacuolization. Leucocytes at 72 hours are very few, and phagocytic cells containing débris are beginning to appear. Occasional normal fibers are seen lying next to badly degenerated fibers at all stages of the muscle injury.

It appears strange that evidence of regeneration should be less conspicuous at 72 hours than at 24 hours after injury. Study of the earlier sections, however, leads to the conclusion that proliferation is started by the muscle nuclei set free by the breaking up of severely traumatized fibers. This proliferation seems to proceed no farther than the sending out of delicate fibrils by individual cells, which are then involved in the progressively deepening muscle degeneration. It seems likely that this first attempt at repair, then, is only a primary response to trauma by nuclei which are too badly damaged to carry on.

At the end of 5 days there are still many large islands of hyalinized fibers within which there is practically no cell infiltration or proliferation. In addition to segmentation and clumping of the cytoplasm in these fibers there now appear in some of them irregular spaces with frayed margins, giving them a moth-eaten appearance. Marked early muscle cell proliferation occurs around the margins of such patches of fiber degeneration. There are numerous

MICROSCOPIC APPEARANCE OF INJURED MUSCLE

Within 4 hours after injury there is considerable interstitial edema with some fibrin in the edema fluid. The blood vessels show moderate dilatation, but no thrombosis is evident. A few extravasated erythrocytes are found here and there, but remarkably few for the severity of the injury. In earlier experiments, in which an unpadded wooden board was used in the production of the injury, considerable hemorrhage was occasionally found. The muscle fibers are extensively damaged. Many are ruptured transversely, some completely through the sarcolemma sheath, others only through the myofibrils, with intact sheath. Complete rupture of the fiber results in a clubbed appearance of the fragments, due to retraction of the sarcolemma and extrusion of sarcoplasm. This knobbed end is of smooth hyaline form. Muscle nuclei in these fibers are usually retracted into the fiber and grouped together.

Various early degenerative changes are found. Some few fibers have lost their cross striations, which are, however, retained in other fibers. Longitudinal fibrils are emphasized and a few small vacuoles are seen. The nuclei of the muscle fibers and sarcolemma sheaths appear to be unchanged. The staining reaction of the tissue shows but little alteration, the fibers with swelling and lost cross striations staining somewhat paler than normal.

Separation of fibers by edema brings into prominence occasional scattered muscle fiber sprouts in some of the sections. These are very small new fibers with pointed tips, many nuclei, and no cross striations. Nauwerck (cited by Craciun³) found marked nuclear division beginning 4 to 6 hours after experimental muscle injury, and interpreted it as the first evidence of repair of that injury. In the present study we interpret the nuclear proliferation seen at this very early stage as evidence of repair, not of the damage due to the experimental procedure, but of muscle fiber degeneration sustained during the ordinary activities of the animal. There are very few such proliferating fibers seen in all the sections studied, so that the nuclear growth is not marked enough or widespread enough to be due to the experimental damage to the muscle. Other workers have also considered similar nuclear groupings as part of a non-pathological process, since they were seen in normal animals. Heiden-

with many nuclei, some of the fibers showing early degenerative changes. The majority of persistent new fibers are formed in groups, and these are larger and more normal in appearance than those seen during the earlier periods of study.

SUMMARY

1. In acute molecular degeneration of striated muscle in rabbits the development of muscle fiber degeneration is a progressive process including edema, fibrillar separation, vacuolization, hyaline change, lumpy disruption, granular change and finally, complete dissolution of the muscle cytoplasm. This is associated with some cell exudation, with a high degree of phagocytic activity, and finally with repair.

2. The course of repair is toward regeneration. The completeness of this process appears to depend upon the destructiveness of the lesion, and not upon the extent or severity of muscle fiber degeneration. If the sarcolemma destruction is not too severe and the stroma remains, these, with surviving muscle nuclei, form the integral factors for muscle restoration. With diffuse tissue destruction scarring results.

irregular single cells with fibrillar extensions, and syncytial sprouts with multiple nuclei. Some of these latter appear within old sarcolemma sheaths. Fibroplastic proliferation is likewise beginning. Numerous phagocytes are present, some invading the degenerated fibers where they are at times arranged around the inside of the sarcolemma sheath, entirely surrounding a central core of degenerated cytoplasm. Such cytoplasm tends to show basophilic staining. There are a moderate number of lymphocytes in the interstitium. The various types of cells appearing in muscle regeneration were studied by Forbus⁵ with the use of vital staining, which aided in proving origin but apparently did not by itself identify the cells better than morphological characteristics.

After 8 to 10 days the types of cells present are unchanged. There is considerable more breaking up of the hyaline fibers, with granular degeneration of the lumpy masses. In the areas of regeneration there is considerable basophilic staining of the degenerated fibers. Reparative effort is prominent, with clumps of new-growing muscle fibers up to 1 mm. or more in length. In the larger of these, longitudinal fibrils appear. Sarcolemma nuclei show proliferation. Occasional empty sheaths are found which, when partially collapsed, show striking resemblance to small capillary buds. A very few capillary buds are present, in contrast to the rich number seen in ordinary granulation tissue.

The 12 day period shows larger masses of more mature muscle fibers, in which definite cross striation appears. There is a gradual diminution in size of the areas of degenerated fibers in this and in each of the succeeding periods. At 16 days a cross-section demonstrates the new fibers in an area of repair, arranged in bundles with endomysial bands between them, forming a very faithful reproduction of the normal muscle picture.

In the 18 and 21 day periods a few areas of almost normal sized new fibers are found. An occasional young fiber is seen with all the nuclei of both the muscle and sarcolemma arranged at the borders and projecting like beads stuck on the surface of the fiber. It seems most likely that this is a fixation phenomenon.

Even at 25 to 28 days small numbers of hyaline degenerating fibers are seen. Scattered areas of newly formed, loose, fibroblastic structure are present in which are seen a few lymphocytes, phagocytes and single muscle cells. There are also isolated muscle fibers

DESCRIPTION OF PLATES

PLATE 35

FIG. 1. 4 hours after trauma. Interstitial edema. Rupture of some fibers. Swelling, loss of cross striation and appearance of longitudinal fibrils in many of the fibers. $\times 170$.

FIG. 2. 48 hour stage. Waxy change of fibers. $\times 325$.

FIG. 3. 5 day stage. Proliferation of fibroblasts, sarcolemma and muscle cells. Early attack of phagocytes on degenerated muscle fibers. $\times 190$.

FIG. 4. 8 day stage. Lumpy disruption and phagocytosis of degenerated fibers. Growth of young muscle sprouts. $\times 190$.

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PLATE 36

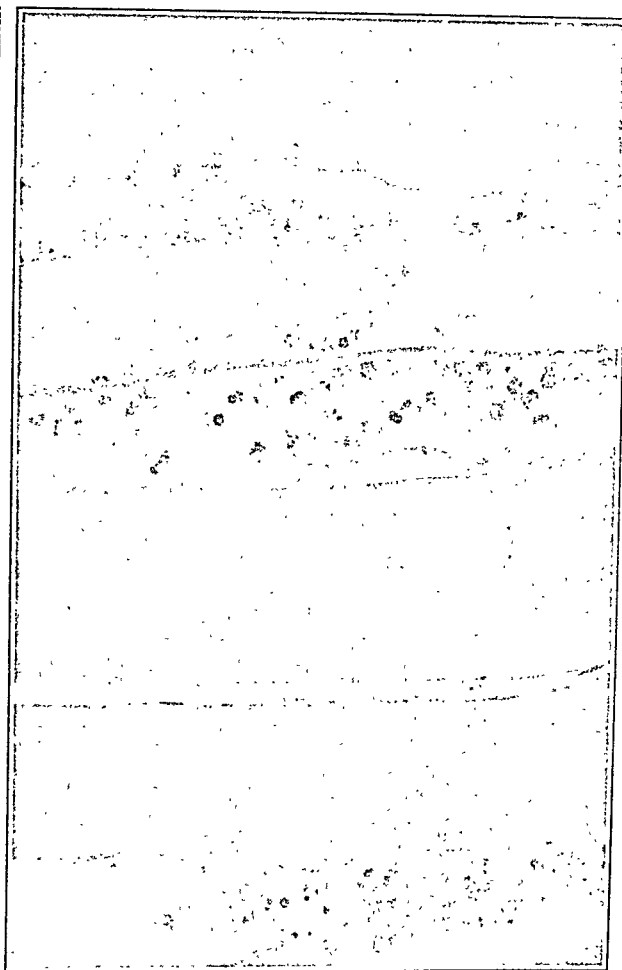
FIG. 5. 10 day stage. New-growing muscle cells connecting old degenerated masses within the sarcolemma sheath. Spindle forms and vacuolated fibers. $\times 190$.

FIG. 6. 12 day stage. New-growing fibers with cross striations appearing. $\times 600$.

FIG. 7. 28 day stage. Regenerated area with fairly normal muscle fiber arrangement. Some excess of interstitium. $\times 37\frac{1}{2}$.



I



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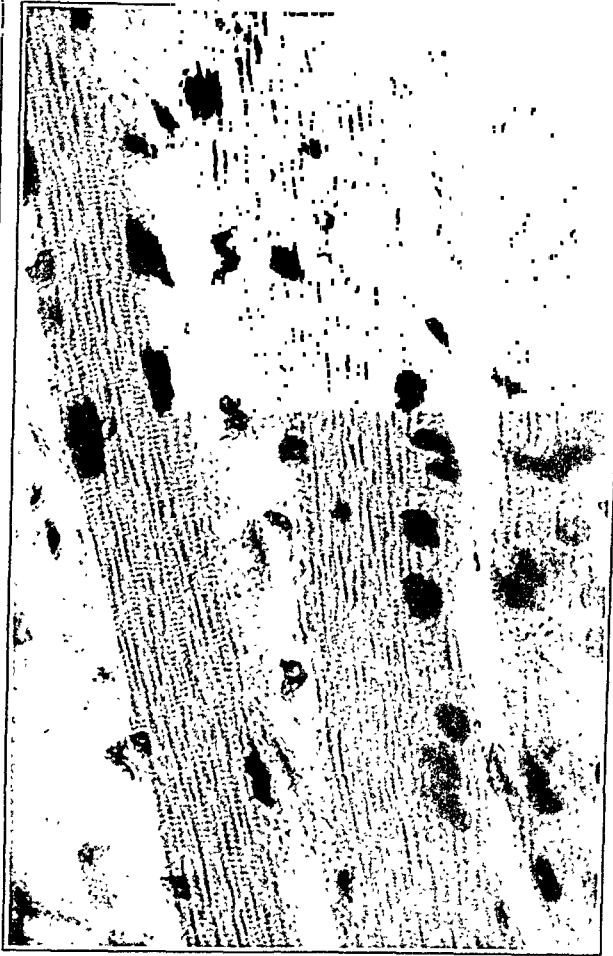
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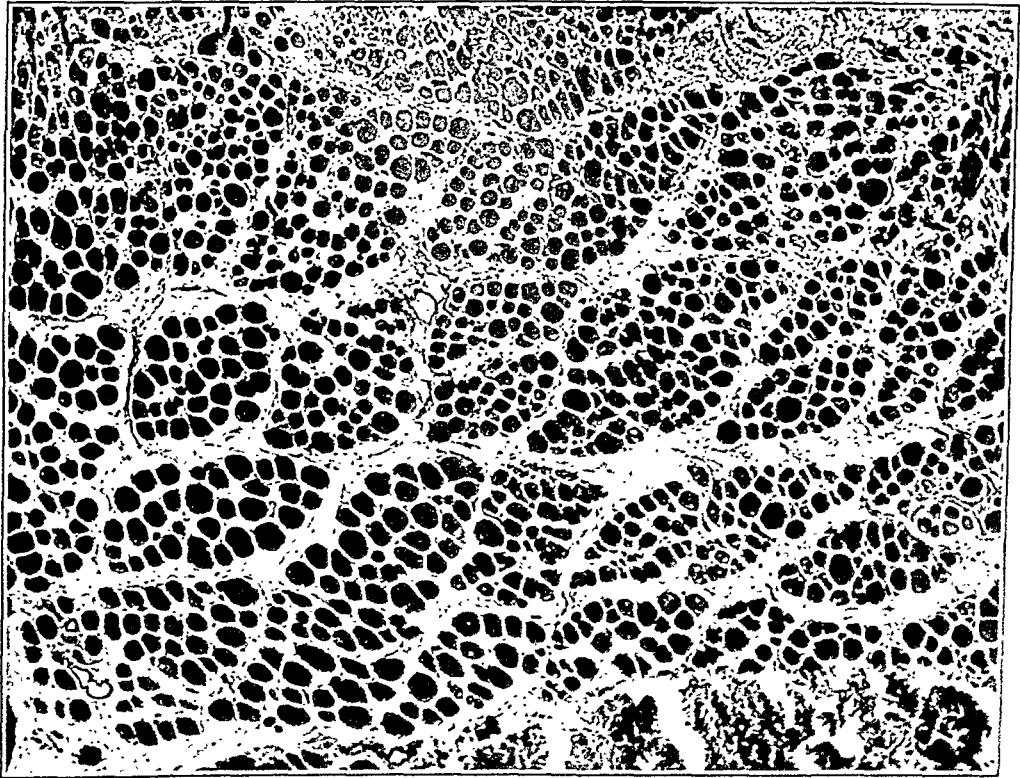
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circulation might furnish an analogy to that of colloidal substances normally present in the blood plasma. Moreover, such studies might demonstrate local variations in the permeability of the intima of the arteries to account for the localization of fatty changes in accordance with the imbibition theory.

The first systematic investigation into the ability of colloidal dyes to penetrate the walls of arteries was made by Petroff⁶ in 1922. He was able to demonstrate staining of the walls of both veins and arteries in the frog's mesentery after intravenous or subcutaneous injection of trypan blue or lithium carmine solutions. The staining, however, was always diffuse. In rats and rabbits, after intravenous or subcutaneous injection of either of these dyes, he observed similar staining of the fine mesenteric arteries and veins. In their aortas he found, in short experiments, marked staining of the elastic lamellae in the inner and outer layers of the media, while the elastic fibres in the intermediate portion remained unstained. In longer experiments, in rats, the staining in the outer layers of the media was always somewhat deeper than that in the inner part. He also observed that application of sodium chloride crystals or weak solutions of hydrochloric acid or silver nitrate to the arteries brought about much deeper and more rapid staining of their treated portions after intravenous injection of trypan blue solution.

Okuneff,⁷ a few years later, carried out experiments on dogs, cats, rabbits, guinea pigs, white rats and white mice, using solutions of trypan blue which were introduced by various routes (intravenously, subcutaneously, intraperitoneally) into the stomach and into intestinal loops. The last two methods of injection were not successful in most of the animals, in that the stain was not absorbed in sufficient quantities to produce visible staining of the aorta. The results from the other three methods of introduction of the dye were very similar to one another but varied considerably in the various animals used. In rabbits, guinea pigs, white rats and white mice, the staining of the aorta was always diffuse. However, in two rabbits and three guinea pigs, small blue patches of rounded outline were observed in the arch of the aorta and usually in its first part. A great similarity existed between the staining of the aortas of dogs and cats. This staining was described as being always fleck-like, although these flecks often coalesced to form larger patches of deep staining. The author called particular attention to the similarity between the distribution of

VITAL STAINING OF THE RABBIT'S AORTA IN THE STUDY OF ARTERIOSCLEROSIS *

G. LYMAN DUFF, M.D.

GEORGE BROWN MEMORIAL FELLOW, UNIVERSITY OF TORONTO, 1931.

(From the Department of Pathology and Bacteriology, University
of Toronto, Toronto, Canada)

With the development of the imbibition or infiltration theory of arteriosclerosis (Ribbert,¹ Aschoff,^{2, 3} Anitschkow^{4, 5}), a new significance has been attached by some investigators to the behaviour of colloidal dyes introduced into the circulation. The relative ease with which the movements of dyes can be traced in the animal body offers particular advantages in the study of colloidal materials in the blood plasma, the properties of which are of special importance in relation to the imbibition theory.

The imbibition theory of "atherosclerosis" depends upon the assumption that fluids may penetrate the intimal surface of the arteries from the lumen. The existence of such an inflow, at least of true solutions, is generally admitted and is looked upon as the normal mode of nutrition of the intima and inner layers of the media. The theory further requires that lipoid materials in colloidal state, having penetrated the intima, may be deposited there under certain conditions, chief among which are a peculiar swelling and loosening of the intima and a sufficient concentration of lipoids, especially of cholesterin esters, in the plasma (Aschoff). The fatty substances are spoken of as being "pressed" into the intima. The experimental production of fatty deposits in the arteries of rabbits fed on high lipoid diets for long periods has been adduced as evidence in favour of the theory. However, in the aortas of both humans and experimental animals, the fatty changes are localized to certain distinct areas which are more or less characteristic for each. Various causes have been assigned for the localization of these deposits and recently the problem has been attacked through experimental studies on the penetration of various colloidal dyes into the walls of arteries. It was hoped that the behaviour of such dyes introduced into the

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inate artery for short periods, separated by intervals in which the clamp was released. In some of the experiments injections of adrenalin and electrical stimulation of the central end of the cut sciatic nerve were also used to increase the blood pressure. He found that the aortas were more deeply stained in those animals in which the blood pressure had been raised, than in control animals in which the same dissection had been carried out but in which the innominate artery had not been clamped. He concluded that an increase of blood pressure could produce a much more pronounced imbibition by the intimal surface of the aorta, not only of dye but also of other colloidal constituents of the plasma.

It appeared to the author that the broad conclusions drawn from the experiments quoted above were in many respects poorly grounded in the experimental evidence. It was felt that further investigation into the question might serve to clarify the situation and possibly provide an adequate explanation for the phenomena observed in vital staining of the arteries. With this object in view, the following experiments were undertaken.

EXPERIMENTS

In this series of experiments only intravenous injection of dye was employed. It was felt that the method of Glasunow was too artificial to be entirely free from objection, while in the experiments of Okuneff no advantage appeared to be gained by intraperitoneal or subcutaneous injection and the dye was not absorbed at all through the normal mucosa of stomach or intestine. Rabbits were used exclusively since, in their arteries, the experimental production of localized fatty deposits is easily accomplished and therefore possible peculiarities in the lining membrane of their aortas should be easily demonstrated. Accordingly, rabbits were injected intravenously with varying quantities of a 1 per cent solution of trypan blue in Ringer-Locke solution, each animal receiving a single injection. In one case a 0.1 per cent solution of the dye was used. The animals were killed at the end of from one hour to one hundred and one hours. The aortas were immediately removed and opened, and the character of the staining in their walls and in other tissues recorded at once. Where the staining of the aorta was irregular the distribution and intensity of the colouring as seen on the intimal surface was recorded

these deep staining areas in dogs and cats and that of the fatty deposits in the aortas of rabbits and in humans. After speaking of this similarity and the probability that common factors were responsible for the typical localization in both cases, he stated: "Nun erweist es sich aus meinen Versuchen, dass auch für eine andere Substanz kolloidaler Natur die gleiche Art des Eindringens in die Aortenwand anzunehmen ist. Die typischen Stellen der Lipoidablagerung in der Aortenwand sind in diesem Fall als Stellen zu betrachten, wo der Durchtränkungsstrom der Blutlymphe am stärksten ausgeprägt ist."

In experiments upon rats, guinea pigs and rabbits, Glasunow⁸ used a somewhat different method. The animal was anaesthetized, the thoracic and abdominal cavities were rapidly opened and a cannula inserted through the left ventricle into the ascending aorta: another cannula was fixed in the vessel just above its bifurcation. The aorta was then perfused with a warmed and oxygenated solution of trypan blue in Ringer-Locke solution for periods varying from five minutes to one hour. In the thoracic aorta a fleck-like distribution of the dye was found. The flecks in the longer experiments coalesced to form dark staining patches, but the colouring was always deepest immediately beneath the mouths of the intercostal arteries, forming crescentic patches which were later joined by longitudinal lines on either side. In the abdominal aorta the staining was more diffuse, but a similar picture was seen about the mouths of the branching vessels. It was found also that short cauterization of the external wall of the aorta or crushing with a clamp resulted in deeper staining in the injured area. Glasunow concluded that the deeply staining areas which he noted corresponded to areas in which the permeability of the intimal surface was greater than elsewhere. It should be pointed out, however, that his method of investigation is open to certain objections. Not only might the normal permeability of the lining membrane of the aorta have been altered under the artificial conditions of the experiments, but also the properties of the dye in association with a simple saline solution might have been very different from those which it would possess in such a complex colloidal system as the blood plasma.

Quite recently, Hackel⁹ has carried out experiments upon rabbits injected intravenously with trypan blue solution. His experiments lasted from thirteen to fifty minutes and during this time he produced an intermittent rise in blood pressure by clamping the innom-

with blue before staining became prominent on the intimal surface of the aorta. In the kidney the staining was considerably deeper in the cortex than in the medulla.

In the aorta blue colouring was first seen in the sinuses of Valsalva, forming a distinct ring around the circumference of the vessel. Staining was also well marked along the borders of attachment of the aortic valves. The valve leaflets were, however, only slightly tinged. The pulmonary valve ring was similarly stained. This staining in both the aorta and pulmonary artery, in the early stages, stopped quite abruptly at the upper margins of the sinuses, while the intimal surfaces of both arteries showed practically no colour. In the longer experiments, where the whole intimal surface of the aorta appeared blue, the aortic valve ring was always prominent by reason of its deeper staining.

The earliest distinguishable colour on the intimal surface of the aorta was always uniformly distributed throughout its whole extent. At this stage it was very obvious that the colour on the adventitial surface of the aorta was distinctly deeper than that on the intimal surface. This difference in the intensity of staining of the two surfaces of the aorta was apparent in all the specimens, but in the longer experiments the difference was not so great.

In the longer experiments lasting sixteen hours or more, the intensity of staining of the aorta as seen from the intimal surface was definitely irregular. The deeper staining patches were not sharply demarcated from the surrounding paler areas, but the colour shaded off from one to the other more or less gradually. Examination of the intimal surface with a strong lens revealed uniform staining within any given small area and the character of distribution of the dye could not be accurately described as "fleck-like." The localization of areas of more intense staining had, in general, a similar distribution in all of the longer experiments; and therefore the aortas from these experiments may be described together concerning the more constant characteristics of distribution of the dye.

The intimal surface of the arch of the aorta always showed more marked irregularities in intensity of staining than elsewhere. A deep staining patch was constantly present, extending from the margin of the anterior sinus of Valsalva upward in the longitudinal axis of the vessel toward the mouth of the innominate artery. This patch was usually broader and deeper in colour at the base, narrowing and fad-

as accurately as possible on "outline charts" of the aorta on which the staining was reproduced graphically with blue crayon. These records were thus available for subsequent comparison.

The following table gives the detail of the experiments.

TABLE I

Rabbit No.	Weight	Dose of trypan blue		Dose per kilo		Duration of experiment	Staining of the aorta as seen on the intimal surface
		gm.	cc. %	cc.	%		
13	1340	4	0.1	3.0	0.1	1	Barely perceptible, staining uniform in distribution
38	1930	8	1	4.1	1	3	
45	2000	10	1	5.0	1	5	
48	1880	10	1	5.3	1	5½	Somewhat deeper staining with a suggestion of irregularity in distribution
49	1500	10	1	6.7	1	5½	
53	1290	10	1	7.8	1	8½	
54	1265	10	1	7.9	1	16	Well marked staining with definite differences in the intensity of colour in different areas. Distribution of variations in intensity were charted
35	1400	10	1	7.1	1	19	
39	2140	8	1	3.7	1	23	
40	2020	8	1	4.0	1	23	
12	1770	5	1	2.8	1	24	
47	2280	10	1	4.4	1	24	
41	2170	8	1	3.7	1	29	
42	2250	8	1	3.6	1	29	
43	1880	8	1	4.3	1	47	
44	1950	8	1	4.1	1	47	
55	1950	10	1	5.1	1	101	

The depth of staining increased with increasing duration of the experiment and appeared to be dependent upon the time rather than upon the quantity of trypan blue, within the limits of dosage employed. It is, however, worthy of note that there was sometimes a considerable variation in the intensity of staining of the aorta in experiments of equal duration, even when comparable quantities of the dye had been injected—a fact which Hackel seems to have overlooked. With increase of the length of the experiments the contrast between deeply stained and lightly stained areas became more marked. However, beyond twenty-three or twenty-four hours, no obvious change in the character of the staining could be distinguished.

Prior to the appearance of blue colour on the intimal surface of the aorta, staining was to be seen in other tissues, as for example in the subcutaneous connective tissue and the peritoneal surface of stomach and intestines. The lungs, liver, spleen and kidneys were also tinged

ence of the aorta but extended slightly further toward the left side than to the right. Its upper limit was approximately the level of the first pair of intercostal arteries, and it extended downward to the level of the diaphragm. In the lower half it was frequently narrower than above and often narrowed gradually to the vanishing point just before reaching the level of the last pair of intercostal arteries.

The abdominal portion of the aorta showed a more uniform staining, though here too the posterior wall of the vessel tended to be more strongly tinged than the anterior. The mouths of the branching vessels were surrounded by narrow zones of paler staining and this was particularly prominent in the distal margins of the vessel mouths forming small crescents of paler staining in these situations.

All the variations in intensity of staining described, with the exception of the pallor around the mouths of branching vessels, could be very easily detected on the adventitial surface of the aorta, being often more distinct on this surface. Cross-section of the vessel and examination of the cut edge with a strong lens showed that the staining was much stronger in the outer layers of the media than in the inner portion. This difference in depth of colour could often be demonstrated even more strikingly by splitting the vessel approximately through the middle of the media and stripping it apart into two layers. The outer layer of the aortic wall then showed well marked staining, while the inner half was almost uncoloured. However, in some of the more deeply stained patches in the arch, the colour extended through the entire thickness of the media to the intimal surface without any perceptible difference in the depth of staining. These findings were confirmed by microscopic examination of thick frozen sections which were mounted without further staining. In such preparations the elastic fibres of the outer portion of the media were seen to be lightly tinged with blue, while the elastic laminae of the remaining part of the media were uncoloured, save for the internal elastic lamina which was faintly tinged. Examination of deep staining patches in the arch showed the elastic fibres to be coloured through the whole thickness of the media, but more strongly so in the outer layers.

In addition to the more or less constant variations in intensity of staining, two specimens (No. 48 and No. 54) showed a single small round patch located on the right side of the ascending limb of the arch about midway between the valve ring and the mouth of the in-

ing in its upper part and thus forming a flame-shaped area. It extended for a variable distance upward but seldom reached the orifice of the innominate artery. Occasionally, however, it split at the upper end, continuing as two more diffuse streaks which passed on either side of the openings of the vessels on the convexity of the arch. Another less distinct patch was almost always present above the left posterior sinus of Valsalva, extending upward for a short distance as a flame-shaped area which gradually faded to the vanishing point. Occasionally this area was extended as a diffuse streak or became broader opposite the opening of the innominate artery, fusing in this area with an extension of the patch first described. The area above the right posterior sinus of Valsalva was always paler than that above either of the other two. The orifices of the great vessels arising from the arch were encircled by a narrow pale staining margin, while darker, mottled areas were often seen near them so that the wall of the aorta on the convexity of the arch was always more deeply stained than that on the concavity. These mottled areas usually resolved themselves posteriorly into a broad streak which extended downward on the posterior wall of the descending portion of the arch toward the openings of the first pair of intercostal arteries. Thus the ascending limb of the arch was most deeply stained on its anterior and left lateral aspects, the colour being deepest at the root of the aorta. In the transverse limb the convexity of the arch and its posterior surface showed the greatest intensity of colour, while in the descending portion the posterior wall was most strongly stained.

The thoracic aorta did not show any patchiness of staining but a distinctly deeper staining of the posterior wall of the vessel was constantly present. This area of deeper staining appeared as a broad streak, often forming a continuation of that coming down from the arch. It was continued laterally a little distance beyond the mouths of the intercostal arteries, and then gradually faded to the paler staining of the anterior half of the vessel. The colour of this area was quite uniform and showed no longitudinal streaking within itself. The only variation in the depth of staining was an extremely narrow margin of pallor just below the mouths of the intercostal arteries. The anterior portion of the thoracic aorta was very lightly stained; indeed, this portion of the aorta showed the weakest staining of any part of the vessel, often appearing almost entirely unstained. This area of pale staining occupied the anterior segment of the circumfer-

irritant had been applied. Microscopic sections of these areas showed, in the case of the animals in which croton oil had been employed, a marked inflammatory reaction in the adventitia and surrounding structures, evidenced by intense engorgement of fine vessels and a moderate cellular infiltration. Unstained frozen sections showed a very faint staining of elastic fibres with trypan blue throughout the thickness of the media, but most marked in the peripheral portion. In the aorta which had been cauterized there was necrosis of the adventitia and outer layers of the media with evidence of an inflammatory reaction about the necrotic tissue. Unstained frozen sections showed the localization of the dye in the necrotic area and diffusely in the regions immediately adjacent to it.

The aortas of three other rabbits were prepared in the following manner. The animal was killed by the injection of air into an ear vein. The thorax and abdomen were rapidly opened and the aorta carefully dissected out. The aorta was immersed in Ringer-Locke solution previously warmed to 37°C and was carefully opened in the longitudinal axis with a fine pair of scissors, care being taken to traumatize the vessel as little as possible. Blood was washed out of the aorta with warm Ringer-Locke solution and the vessel then placed in a weak solution of trypan blue in Ringer-Locke solution also warmed to 37°C and kept at that temperature in an incubator. In one experiment the strength of the trypan blue solution was 1:10,000. At the end of thirty minutes the vessel was quite deeply stained. The two other aortas were immersed in a 1:20,000 trypan blue solution, one for fifteen minutes and the other for twenty-five minutes. In both of them the staining was of moderate depth and comparable to the strength of staining in the longer experiments with intravenous injection of the dye. In all three aortas the staining was approximately of equal intensity on both the adventitial and intimal surfaces. The depth of colour was uniform throughout the whole extent of the vessel, except for a few pale areas where surfaces had apparently come into apposition with one another and interfered to some extent with the free access of the dye. Also a narrow zone along the cut edges was more deeply stained. However, the characteristic distribution of the dye, as described above for the experiments where intravenous injection had been used, was entirely lacking. Microscopic examination of unstained frozen sections also indicated that

nominate artery. In one case (No. 48) the experiment had been of only five and one-half hours' duration and this area was quite deeply stained, while the remaining parts of the intimal surface showed only slight staining. These patches were much less prominent on the adventitial surface. On sectioning these areas no abnormality was found in the structure of the wall and the staining was most marked in the layers of the elastic fibres nearest the intima, gradually fading toward the periphery.

In four of the animals one or more "spontaneous" arteriosclerotic lesions of the aorta were present as small, discrete, slightly depressed areas in the arch or the upper part of the thoracic aorta. In every instance these lesions were conspicuous because of the almost complete lack of colour in them. They stood out as small, round, white patches against the blue background. There was no greater intensity of staining around the margins of the lesions than elsewhere in the intima. A patch of slightly deeper staining was, however, to be seen on the adventitial surface opposite each of them.

The pulmonary artery became stained in about the same time that was sufficient to produce perceptible staining of the aorta. The intimal surface of the first part of the pulmonary artery was often mottled in appearance, but variations in intensity were not as marked as in the arch of the aorta. The smaller arteries and veins were also coloured, at least in the longer experiments, and in these, as far as could be determined, the staining was uniform. The thoracic duct, if it was stained at all, was not sufficiently coloured to make it any more conspicuous than usual.

In addition to the experiments described above, three rabbits were anaesthetized and the abdominal aorta exposed by a transperitoneal approach. In one animal the aorta was painted with croton oil around its whole circumference for a distance of about 1 cm. In another only a portion of the external surface of the aorta was bared and painted with croton oil. In the third, the aorta was lightly seared along one side with a hot probe. Immediately after operation in each case the rabbit was given 10 cc. of 1 per cent trypan blue in Ringer-Locke solution intravenously, and killed five hours after the injection. In each animal the aorta was lightly stained throughout its extent, except in the region treated with croton oil or cautery, where both the adventitial and intimal surfaces showed a patch of much deeper blue-staining, corresponding to the area in which the

pan blue demonstrated that the irregularities of staining in the aortas of animals injected intravenously with the dye were not due to the presence of unusually spongy tissues or to areas possessing a greater affinity for the dye. They indicated, on the contrary, the importance in the staining process of the maintenance of normal blood flow and pressure, not only in the lumen of the aorta, but also in the vasa vasorum.

Impressed by the importance of blood pressure, particularly in the lumen of the aorta, Okuneff, Glasunow and Hackel attempted to establish that the staining is due to penetration of the dye directly from the lumen of the vessel into the intimal surface. They believed that the local variations in intensity of staining could be explained by corresponding variations in the permeability of the lining endothelium which, in turn, was looked upon as being dependent upon variations in the strength of the normal lymph flow from the lumen of the aorta to the lymph channels in the peripheral coats of the vessel. If this were the case, one would expect to find the innermost layers of the aorta stained earliest and most deeply. It is true that in these experiments the internal elastic lamina was lightly tinged by the dye, indicating that some penetration from the lumen did occur, but the staining of this membrane was always diffuse and completely overshadowed by that of the external layers of the vessel wall. It was in the latter that the variations in intensity of staining occurred which produced the irregularities in depth of colour seen on the intimal surface.

From an anatomical study of the aortas of dogs, lambs and humans, Robertson¹⁰ has minutely described the distribution and abundance of the blood supply to the walls of the arch and thoracic portion of the aorta, through the fine vessels which ramify in the adventitia and penetrate the outer third of the media. A comparison of his results with those of the present experiments shows a close correspondence between the depth of staining in the aorta and the vascularization of its walls: the colouring is deepest where the vascular network is most abundant. He found a rich network of vessels about the aortic valve ring and the root of the aorta, but fewer in the ascending limb of the arch. Furthermore, the vascular supply was not equally plentiful around the whole circumference of the vessel. "At the root of the aorta, vascularization was most abundant over the anterolateral aspect and least abundant behind, over the right pos-

the staining was of a different character. The elastic fibres in the inner and outer thirds of the media were tinged with blue, while the middle third of the media was entirely unstained.

DISCUSSION

In considering the picture of dye distribution, as seen on the intimal surface of the aorta, one must keep in mind that the aorta of the rabbit is quite translucent and that staining of the adventitia or external layers of the media is clearly visible on the intimal surface of the vessel. This fact can easily be demonstrated by placing a drop of 1 per cent solution of trypan blue on the adventitial surface of a freshly removed aorta and, after a few moments, washing it off and opening the vessel. The situation of the stain in the adventitia can be detected with ease from examination of the intimal surface.

The results of the present experiments indicate that the colour seen on the intimal surface of the rabbit's aorta after intravenous injection of trypan blue is due to dye which is confined chiefly to the adventitia and outer portion of the media or which diffuses inward from this source. This is evidenced by the earlier appearance of colour in the adventitia and by the fact that the former is more deeply tinged, especially in the shorter experiments. Also the variations in intensity of staining seen on the intimal surface of the vessel are equally well marked on its external aspect. Splitting of the aortic wall approximately through the middle of the media and stripping it apart into two layers shows strong staining of the outer half, while the inner layer is almost uncoloured. Furthermore, examination of cross-sections of the aorta, both in the gross and microscopically, shows the presence of the dye in the adventitia and outer portion of the media while the inner layers are relatively unstained. In some of the very deeply stained parts of the aorta, the dye has tinged the whole thickness of the media, but even in these areas the staining is stronger in the external layers.

In view of these facts, any explanation of the variations in intensity of staining, as seen on the intimal surface of the aorta, must be based upon local peculiarities in the structure or function of the adventitia and peripheral third of the media. The experiments in which freshly removed aortas were soaked in weak solutions of try-

plained by the local increase of capillary permeability in the inflammatory reaction. That the permeability of the capillary walls to trypan blue does increase in an area of inflammation has been demonstrated by Menkin^{12, 13, 14} in experiments upon rabbits and frogs. He also showed that the dye injected into the blood stream rapidly accumulated in an area of inflammation and was fixed there by occlusion of the regional lymph channels and the formation of a network of fibrin around the inflamed area.

Since the greater part of the blue colour seen in the intimal surface of the aorta was imparted to it by the outer coats of the vessel, any local thickening or density of the medial coat or intima would result in an area of relative pallor. Thus a narrow zone immediately below or completely encircling the orifice of each branching vessel appeared paler in colour, due to the presence of a lip-like thickening around the mouth of each arterial orifice, particularly prominent around the distal margin. The failure of "spontaneous lesions" to show any colour on the intimal surface probably likewise depended upon the density of the areas of degeneration in the media which frequently showed calcification. On the other hand, the slightly deeper staining on the adventitial surface opposite such lesions probably was the result of an increased vascularity in response to a degeneration and mild inflammation of long standing.

From the results of the present experiments, conclusions such as have been drawn by Okuneff, Glasunow and Hackel would be quite unjustified. The irregularities in intensity of staining of the aortic wall cannot be said to be due to local variations in the permeability of its lining endothelium. If the behaviour of trypan blue be comparable to that of lipid materials in the plasma, then one must conclude that local variations in the permeability of the intima to lipoids do not exist under normal conditions, and hence can have no bearing upon the initiation of local fatty deposits in the aorta. The explanation of the origin and localization of such fatty changes must be sought elsewhere.

CONCLUSIONS

1. In rabbits, intravenous injection of a suitable quantity of a solution of trypan blue results in well marked staining of the wall of the aorta within sixteen hours. The depth of colour as seen on the intimal surface is not uniform, some areas being more deeply stained

terior sinus of Valsalva. . . . The vascularity of the arch was greatest on the convex surface of its ascending portion, and on the posterior surface of its transverse portion. It was least vascular on its anterior surface, particularly toward its descending portion. This latter section was most vascular on its posterior aspect, resembling the descending thoracic limb in this respect." The thoracic portion of the aorta was most richly supplied on its posterior wall, while the anterior segment had a much less abundant vascular network. The similarity between the position of these areas of abundant vascular supply and that of the areas most deeply stained by trypan blue in the present experiments need hardly be further enlarged upon to indicate the probability that the distribution of the dye was dependent upon the abundance of the vasa vasorum in the adventitia and outer third of the media. Where the vascular network was sufficiently abundant the stain was diffused through the thickness of the media, even reaching the inner surface. Apparently the endothelium of the capillary network was much more permeable to the dye than was that of the intimal surface of the aorta and the large vessels. This fact resulted in the appearance of the dye in other tissues, as well as in the external coats of the aorta, at a time when the intimal surface of the aorta was still almost uncoloured.

In two rabbits (No. 48 and No. 54) a small round patch was found on the right side of the ascending limb of the arch of the aorta and in these areas the staining was found to be most marked in the inner layers of the media. These patches probably marked the position of minute nutrient vessels entering the media directly from the lumen of the aorta and ramifying in its inner portion. The presence of such vessels in this same position in dogs has been reported by Woodruff ¹¹ and by Robertson, and the latter also found perforating nutrient vessels of this type in human aortas. In some cases these vessels anastomosed with the network in the outer coats of the aorta, while in others they ended in a ramification in the inner third of the media. The occurrence of such minute vessels probably accounts for the patches observed by Okuneff in the aortas of some of his rabbits and guinea pigs.

An inflammatory reaction induced in three rabbits around a small portion of the abdominal aorta through the agency of croton oil or cauterization resulted in earlier and stronger staining in the inflamed areas than elsewhere in the vessel wall. This phenomenon is readily ex-

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than others. The differences in intensity of staining become more prominent with increase in the length of the experiment.

2. The variations in depth of colour seen on the intimal surface of the aorta are the result of irregularities in the staining of its outer layers — the adventitia and outer portion of the media. The deeply staining areas correspond to the areas in which the aortic wall is most plentifully supplied by vasa vasorum, while the pale staining areas correspond to those in which the vascularization of the aorta is least abundant.

3. The staining of the wall of the aorta is chiefly due to the escape of the dye through the capillary endothelium which is much more permeable to trypan blue than is the lining endothelium of the aorta. The local variations in depth of staining in the aorta are thus dependent upon the degree of vascularization of its walls.

4. The production of an inflammatory reaction in the external layers of the aorta brings about a local increase in capillary permeability to trypan blue and as a result a stronger staining of the vessel wall in the inflamed area.

In conclusion I wish to express my thanks to Professor Oskar Klotz for his interest and advice throughout the progress of this work.

EXPERIMENTAL PROCEDURE

The rabbits in the experiments reported in this paper were, at the outset, placed on a cooked cabbage diet, as it was anticipated that a shorter period of feeding would be required to develop goiters.

The feeding was started in January 1931, with 17 rabbits. White winter cabbage was used. That used for the first two months was grown in New York state. For the first month the animals were fed 35 gm. whole oats weekly, 20 gm. hay weekly, and as much cooked cabbage as they would eat.

Four animals died during this period after losing weight rapidly. Three of these animals showed hemorrhagic lungs at autopsy.

After the first month, as the animals were not gaining weight on this diet, the cabbage of each rabbit was weighed daily, and the oats increased to approximately 50 gm. The cabbage was steamed in the autoclave (at first under pressure, but later no pressure was used) and was given in the amounts indicated in Table I. The weights recorded are for the cooked cabbage after steaming. The animals ate voraciously and seemed hyperactive, and when excited sometimes showed protrusion of the eyes. As much cabbage was given to the animals as they would eat, and from the table it will be seen that the amount eaten was much more than was given by either the Johns Hopkins investigators or by Marine and his co-workers. However, only 5 animals maintained their original weight, as may be seen from the table, and these 5 did not gain as much as a rabbit would on a regular diet.

At the beginning of the third month, as the animals were still doing poorly and showed no palpable thyroid enlargement, they were divided into two groups, including an equal number of rabbits who were losing weight in each group. This division is indicated in the table as "2nd period" of the experiment.

One group was fed cooked cabbage from which the juice had been expressed in a press. Marine and his co-workers⁶ had shown that the juice does not contain the goiterogenic agent, and the pressed cabbage is just as effective as the whole. The cabbage was pressed in order to reduce the polyuria which rabbits show on a cabbage diet. It was thought that this increased water intake might be a factor in producing the poor nutritional state of the animals. As much of this pressed cabbage was given each animal as it would eat, and the

THE EFFECT OF CABBAGE FEEDING ON THE MORPHOLOGY OF THE THYROID OF RABBITS *

ISOLDE T. ZECKWER, M.D.

*(From the Department of Pathology, University of Pennsylvania Medical School,
Philadelphia, Pa.)*

The production of goiters in rabbits by cabbage feeding reported by Chesney, Clawson and Webster,¹⁻⁵ and confirmed by Marine, Baumann and Cipra⁶ seemed to introduce an easy and certain experimental means for studying thyroid hyperplasia in rabbits. Although these goitrous rabbits showed heat production lower than normal,² yet when iodine was administered to such rabbits there was a striking rise in basal metabolic rate, deposition of colloid, loss of weight and sometimes death.

It was for the purpose of studying carbohydrate metabolism during the hyperthyroid state induced by the administration of iodine to such goiter-bearing rabbits, that a group of rabbits was placed on a cabbage diet.

The diet used by the Johns Hopkins investigators was a daily ration of approximately 250 gm. of cabbage, and a weekly ration of approximately 20 gm. of hay and 50 gm. of oats.⁵ The diet used by those working at Montefiori Hospital was 60 calories of cabbage per Kg. daily (equivalent to about 180 gm. cabbage per Kg.), 35 gm. whole oats weekly, and 20 gm. alfalfa hay weekly.⁷

Marine, Baumann and Cipra⁶ had found that boiling or steaming the cabbage greatly increased its capacity to produce hyperplasia. Whereas rabbits fed fresh cabbage developed palpable thyroids in about 30 days, the same amount of steamed cabbage produced an equivalent enlargement in 10 to 15 days. Steamed cabbage in amounts as low as 25 calories per Kg. (or about 75 gm. per Kg.) per day produced hyperplasia. They considered that cabbage contained a "powerful goiterogenic agent."

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Marine⁷ reported externally palpable thyroids and enlargements up to two and two and one-half times on direct observation in 4 rabbits fed 81 days on raw winter cabbage (1928); palpable thyroids and enlargements of three to four times in 4 rabbits fed 56 days (1928); palpable thyroids and enlargements of twice normal size in 8 rabbits fed 28 days (1928); and palpable thyroids and enlargements of three times normal size in 2 rabbits on the diet 22 days (spring of 1929). During the fall and winter of 1929-1930 he reported that cabbage was less goiterogenic, that is, the thyroids could not be palpated after 21 days of feeding. These figures were for raw cabbage.

Comparison of our data with that of previous investigators shows that under the conditions of the experiment reported in this paper 1931 cabbage produced very little gross enlargement of the thyroid. The three heaviest thyroids were obtained in rabbits which had been transferred from a cooked cabbage diet to raw cabbage.

3. *Microscopic Appearance of the Thyroid*: In the papers from Johns Hopkins,^{1,3} microscopic descriptions have been given of the goiters in a stage of advanced hyperplasia, and descriptions have been given of the microscopic changes of involution induced in these enlarged thyroids by iodine administration. The early stages in the formation of the goiter, however, were not described. In the present paper, therefore, the microscopic changes will be considered in some detail, as they apparently represent the early stages in the development of the type of goiter which results from a cabbage diet up to the stage at which the other observers began their microscopic studies.

Although the gross changes in the thyroid were not striking in the present series of experiments, the microscopic changes were pronounced and represented an interesting transition from the normal to the hyperplastic.

The microscopic slides were arranged in order of what was considered the degree of microscopic change, without referring, until after the histological description, to the data of weights and duration of thyroid feeding. In Table I the rabbits have been arranged in this order, ranging from Group I in which the microscopic changes were slight, to Group III in which the microscopic changes appeared to be the most advanced in this series.

amounts are recorded in Table I. The weights recorded are of the cabbage after expressing the juice, corresponding roughly to 0.7 of the original weight of the cabbage. The second group was fed raw cabbage.

At Montefiori Hospital all of the rabbits were of a single strain (Belgian). At Johns Hopkins various types of rabbits were used. In the experiments reported in this paper various types were used.

RESULTS

1. *Condition of Animal:* It will be seen from Table I that 6 animals at the end of their feeding periods had maintained their original body weights, and of these, 3 had exceeded their original weights. Eleven animals were below their original weights, either at spontaneous death or when the experiment was intentionally terminated. The animals studied by Chesney, Clawson and Webster¹ usually gained weight and had no tendency to diarrhea. Some of their animals, however, lost weight rapidly for several weeks and died, the autopsy revealing no cause for death. Webster and Chesney⁵ reported a high incidence of intercurrent infection at times. The animals of the present series, which lost weight rapidly, were suffering from diarrhea. Five animals died spontaneously, and 2 were killed because they were ill. In 4 ill rabbits, there were acute inflammatory lesions in the lungs. The rabbits used were not a parasite-free breed, and coccidia were sometimes found at autopsy.

2. *Weight of Thyroid:* The only thyroids in the present series that were distinctly larger than normal were one of 0.641 gm. after 102 days of feeding (R73), and one of 0.4398 gm. after 107 days (R64). Comparable weights were obtained by Chesney, Clawson and Webster¹ in much shorter periods of time. For instance, they obtained 0.86 gm. as the average weight for all rabbits observed for 41 to 60 days, and 0.43 gm. as the average of all animals observed for 21 to 40 days. Their data for periods of observation comparable to mine show that at 101 to 120 days the minimum weight of the thyroid was 0.4 gm., the maximum 3.0 gm., and the arithmetical mean 1.47 gm. For 81 to 100 days they obtained a weight of 0.1 gm. as a minimum, 2.3 gm. as a maximum, and 1.05 gm. as an arithmetical mean.

TABLE I
Showing Slight Microscopic Changes)

TABLE I Data on Cabbage-Fed Rabbits. (Group I, Showing Slight Microscopic Changes)												Condition of animal
Animal No.	Body weight, Ks.		No. days	Cabbage feeding			Weight gm.	Gross	Thyroid		Microscopic	
	Original wt.	Final wt.		Approximate No. gm. per day	Condition of cabbage	Gross			Thyroid			
										Original wt.		
R51	1st 2nd	2.68 2.48	2.48 2.32	70 43 113	450-700 500-700	Cooked, not pressed Raw	0.1284	Small, pale	Epithelium flat and cuboidal. Colloid acini large, a few small. Many abundant and mostly dense		Snuffles during first month. Steady loss of weight throughout experiment	
R59	1st 2nd	2.38 2.10	2.10 2.12	70 44 114	450-700 400-700	Cooked, not pressed Steamed, pressed	0.1010	Small, pale	Epithelium becoming cuboidal. Many small acini. Colloid abundant and dense	Good condition		
R58	1st 2nd	2.34 1.98	1.98 1.50	70 39 109	450-700 450-600	Cooked, not pressed Steamed, pressed	0.1655	Small, vascular	Epithelium flat and cuboidal. Some between acini. Increase in cells between acini, with crescents	Killed because ill. At autopsy, intestine contained no solid feces. Coccidia		
R62	1st 2nd	2.10 2.02	2.02 2.06	63 44 107	400-700 400-700	Cooked, not pressed Steamed, pressed	...	Very small, congested	Epithelium flat and cuboidal. Many small acini. Colloid granular, with crescents	Found dead. Left lung hemorrhagic. Intestine contained no solid feces. Coccidia		
R61	1st	2.44	1.50	36	600	Cooked, not pressed	...	Very small, pale	Epithelium flat and cuboidal. Small and medium sized acini. Colloid pale but homogeneous and has escaped outside acini	Found dead. Hemorrhagic lungs. Blood-stained fluid in chest and peritoneal cavities		
R66	1st	2.04	1.50	33	ad lib	Cooked, not pressed	...	Small, slightly congested	Mostly flat epithelium. Scanty granular colloid, some outside acini	Found dead. Hemorrhagic lungs. Blood-stained fluid in chest and peritoneal cavities		
R65	1st	2.66	2.26	17	ad lib	Cooked, not pressed	...	Small, slightly congested	Mostly flat epithelium. Scanty granular colloid, some outside acini	Found dead. Hemorrhagic lungs. Blood-stained fluid in chest and peritoneal cavities		
R60	1st	2.44	2.24	24	ad lib	Cooked, not pressed	...	Small, slightly congested	Mostly flat epithelium. Scanty granular colloid, some outside acini	Found dead. Hemorrhagic lungs. Blood-stained fluid in chest and peritoneal cavities		

Group I. Early Changes: The essential changes were thinning and disappearance of the colloid, change in shape of the epithelial cells, and increase in number of the epithelial cells, with formation of new small acini. In some thyroids the change in the epithelial cells far exceeded the disappearance of colloid. That is, the former flat epithelial cells became oval and rounded, even when enclosing a large amount of fairly dense colloid which, however, had become paler than normal. Where the colloid was most dense and eosinophilic, the cells remained very flat. In addition, there were solid masses of round and oval epithelial cells with abundant cytoplasm lying in between large acini and grouped together without apparent formation of acinar spaces. These changes are exemplified in R51 and R59 (Fig. 1).

In R58 there was more marked change in the colloid than in the epithelial cells. The colloid had become pale and granular. Apparently in life this colloid was quite fluid, as it seemed to have sedimented by gravity, and had thus formed crescents of eosin-staining material in each acinus with an upper zone of granular, poorly staining material, while above this the acinus was empty. There was relatively little rounding of the epithelial cells, but there was some new-formation of epithelium between old acini.

In other cases (R62, R61, R66) thinning and disappearance of colloid occurred almost simultaneously with rounding of the cells and new-formation of epithelial cells. Sometimes a few acini containing dense eosinophilic colloid stood isolated, surrounded by acini containing pale, fluid colloid (Fig. 2). Whenever there was evidence of thinning of colloid, dense crescents were found in the acini with granular colloid above. The acini became somewhat smaller in size and new epithelial cells appeared between acini. The changes in this group were only slightly beyond physiological variations, but in no instance was the "resting" colloid stage seen.

In 2 of the rabbits that died in less than a month (R65 and R60) the microscopic appearance did not correspond with the sequence of changes in the other thyroids, and possibly other factors complicated the picture. In these, pale colloid not only distended the acini but occurred in lakes outside the acini. There appeared to be ruptures in the acinar walls, permitting this flowing out of colloid into the interstitial spaces.

Group II. Changes of Moderate Degree: The thyroids of this group showed acini of moderate size and very small size. The epithelial cells were distinctly cuboidal and there was extensive hyperplasia, as indicated by the dense masses of round cells without discernible lumina lying in between small, apparently new-formed acini. The epithelial cells of the moderate sized acini were very prominent because of the large amount of cytoplasm and clear round nuclei. The colloid was very pale and granular and generally greatly reduced in amount (see Fig. 3 in which the histological changes were quite advanced in a small thyroid).

Group III. Changes of Advanced Degree: In the three heaviest thyroids the epithelial cells were high cuboidal, with a large amount of pale, vacuolated cytoplasm. Most of the cells occurred in dense groups forming very small acini. The colloid was scanty and when present was pale and granular. Capillaries were conspicuous. Fig. 4 represents the most advanced change, which has the appearance of the stage studied by Chesney and Webster.

DISCUSSION

Apparently there is very wide variation in the way in which rabbits respond to cabbage diet, either by microscopic hyperplasia or gross enlargement of the thyroid. Webster and Chesney commented upon individual susceptibility of certain rabbits to the goiterogenic agent, and noticed that goiter was more easily produced during the winter. Just after the present experiment was started, Webster, Marine and Cipra⁷ published results showing seasonal variation in the goiter produced by feeding of cabbage, and variation from year to year, that is—1929 cabbage was not as effective as 1928, and 1928 not as effective as 1927. Their results are stated as the beginning of a systematic attempt to study seasonal variations. The present experiments, although limited in number, may add further data indicating annual variation and a failure to produce thyroid hyperplasia of high grade by feeding winter cabbage during the early part of the year 1931, under the conditions of the experiment.

McCarrison⁸ has recently reported obtaining goiter in rabbits by cabbage feeding in India. On examining his figures, it is seen

(Group II, Showing Microscopic Changes of Moderate Degree)

	1st	2.16	1.72	24	ad lib	Cooked, not pressed	...	Very small, slightly congested	Epithelium cuboidal. Very cellular areas where lumina not discernible. Scanty granular colloid	Found dead. Intestine contained no solid feces
R71	1st	2.16	1.72	24						
	2nd	2.30	1.90	70 24 94	450-700 450-500	Cooked, not pressed Steamed, pressed	0.2520	Pale	Cells cuboidal. Very little new formation of acini. Scanty granular colloid	Found dead. Lungs consolidated, pus in pleural cavity
R69	1st	2.18	2.26	63 39 102	400-700 600-700	Cooked, not pressed Raw	0.1415	Pale	Cells all cuboidal. Large acini and new small acini. Colloid granular	Good condition
	2nd	2.26	2.24							
R67	1st	1.96	2.00	63 42 105	400-700 400-600	Cooked, not pressed Steamed, pressed	0.0882	Vascular	Cells cuboidal. Large acini as well as new small ones. Colloid granular	Good condition
	2nd	2.00	1.96							
R72	1st	2.44	2.06	63 38 101	400-700 600-700	Cooked, not pressed Raw	0.1630	Pale	Cells cuboidal. New acini. Granular colloid with crescents	Loss of weight. Coccidia
	2nd	2.06	1.96							
R68	1st	2.42	2.44	63 40 103	450-700 400-600	Cooked, not pressed Steamed, pressed	0.1834	Pale	Cuboidal cells. New formation of many small acini. Colloid varies from dense to granular	Good condition
	2nd	2.44	2.58							

(Group III, Showing Microscopic Changes of Advanced Degree)

	1st	2.10	1.82	63 40 103	400-700 600-700	Cooked, not pressed Raw	0.2720	Extremely cellular. High cuboidal epithelium. Most acini small. Granular colloid where present	Good condition
R63	1st	2.10	1.82	63 40 103	400-700 600-700	Cooked, not pressed Raw	0.2720		
	2nd	1.82	1.80							
R64	1st	2.30	2.28	63 44 107	400-700 500-700	Cooked, not pressed Raw	0.4398	Extremely vascular	Extremely cellular. Mostly small alveoli with high cuboidal epithelium. Very little colloid	Good condition
	2nd	2.28	2.28							
R73	1st	1.92	2.26	63 39 102	400-700 600-700	Cooked, not pressed Raw	0.641	Extremely vascular	High cuboidal epithelium. All acini small. Almost no colloid. Capillaries conspicuous	Good condition
	2nd	2.26	2.36							

DESCRIPTION OF PLATE

PLATE 37.

- FIG. 1. Very little histological change in R59 after 114 days of feeding. Thyroid weighed 0.101 gm. $\times 560$.
- FIG. 2. R62. Cells are generally low cuboidal. Colloid in some acini is becoming thin and granular, in contrast to a few acini containing dense eosinophilic colloid surrounded by flattened cells. Thyroid weighed 0.1655 gm. 107 days' feeding. $\times 560$.
- FIG. 3. R67. Cells are high cuboidal, many acini are small, and the little colloid that remains is granular and pale. Thyroid weighed only 0.088 gm. 105 days' feeding. $\times 560$.
- FIG. 4. R73. This represents the most advanced hyperplasia noted, in the heaviest thyroid of the series. The cells are high cuboidal, acini very small, and the colloid has largely disappeared. The thyroid weighed 0.641 gm. 102 days' feeding. $\times 560$.

that the degree of hyperplasia was not very great, the heaviest thyroid obtained weighing 0.697 gm.

The experiments reported in the present series are few in number because intended for another purpose, and conclusions must therefore be guarded, and yet in the findings reported in previous years the regularity with which hyperplasia resulted was such as to anticipate hyperplasia in a large percentage of a small series. When the results became apparent, it was no longer possible to obtain winter cabbage of the same year, and therefore extension of the experiments to a larger series was impossible.

SUMMARY

1. Feeding winter cabbage in the early part of 1931 to 17 rabbits for periods up to 114 days produced hyperplasia of the thyroid, but only in two instances resulted in enlargements more than twice the normal weight.

2. The microscopic changes of hyperplasia were more conspicuous than the gross enlargement.

3. Under the conditions of these experiments, the feeding seemed to favor a high incidence of intercurrent infections.

4. The data, in so far as a small series of experiments permit conclusions, support the view that there is annual variation in the goiterogenic agent of cabbage.

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Zeckwer

Effect of Cabbage Feeding on Thyroid of Rabbits

was found to be of exceptional value in sharpening the details of the impregnation and converting it essentially into a double impregnation. There are six variants of our procedure chosen from our experimental series of thirty-six variants. The reader need not be alarmed at this large number of variants: it is intended that they shall be used to fit the case in question and ample indication will be afforded for the choice of the proper one, with a tabular view of the results obtainable with each. Unless the best one for a particular purpose be chosen, the results will not be optimal, although any one of the six will give pictures superior to those obtained through methods heretofore used for demonstrating the finest fibrils of the connective tissue. The method is simple, counterstaining is entirely eliminated, and every detail of a given tissue may be brought out sharply.

TECHNIQUE

Fixation: The finest results obtained were seen in sections made from material fixed in formalin and kept as museum specimens in Kaiserling III for nearly ten years. This fixation, however, is scarcely to be considered practical. The next best fixative is neutral 10 per cent formalin, in which blocks cut thin enough to ensure complete penetration of the fluid should remain for 24 hours at least, longer if possible. If Bouin's fluid is used, the results are comparable to those obtained in the Laidlaw-Bouin method; the nuclei will be unimpregnated, the cytoplasm impregnated in the case of epithelial cells, and mesoblastic cells will be unstained. The resulting pictures are more colorful than those obtained by the Laidlaw procedure.

The method gives very good results if Zenker-fixed tissues are used. They should be fixed for 24 hours, washed in running water for another 24 hours and, after embedding and sectioning, the mercuric chlorid should be removed from the sections with the usual alcoholic iodine solution, and this in turn removed with very weak (1 per cent or less) aqueous sodium thiosulphate. This must then be washed out thoroughly. The oxidation-reduction steps, in which potassium permanganate and oxalic acid are used, *should be omitted* as they produce effects similar to the Bouin fixation. The presence of chromium salts makes no material difference in the subsequent impregnation, except to enhance the impregnation of nervous tissue. On the whole,

A TECHNIQUE OF SILVER IMPREGNATION FOR GENERAL LABORATORY PURPOSES *

NATHAN CHANDLER FOOT AND ELLEN BELLOWS FOOT

(From the Department of Pathology, College of Medicine of the University of Cincinnati, and the Cincinnati General Hospital, Cincinnati, Ohio)

Anyone who has used silver impregnations over a period of time will have been struck with the possibility of applying them to the demonstration of histological elements other than fibers, and will have been tempted to devise some method that would, at one and the same time, bring out these structures as well as the fibrous elements in a given section. There seems to be no reason why a silver impregnation should not be arranged to fit the purposes of routine tissue examination in the pathological laboratory, a method that would be an improvement over the usual routine stains, inasmuch as it would demonstrate a variety of tissue elements selectively without rendering the use of several stains on several sections necessary.

The following method was designed primarily to demonstrate the finer fibrils in tumors of the melanoma group which elude silver impregnation when the usual methods are employed. They could be shown in frozen sections, but only partially or unsatisfactorily brought out in paraffin material. We therefore experimented with a series of some thirty-five different modes of procedure and discovered that it is possible to obtain even better results in paraffin than in frozen sections. While doing this, we were struck with the general applicability of the method to the demonstration of other tissue components as well. It was found that the preliminary bleach with potassium permanganate and oxalic acid, used in prevailing methods for impregnating reticulum and endoneurium, was the stumbling block that had obstructed successful impregnation of the finer fibrils of our tumors. Further experimentation demonstrated that a preliminary treatment with pyridin and glycerol, in place of the bleach, was practically essential for the attainment of satisfactory results and, in subsequent work, the use of a reducing agent following the gold toning bath (as suggested by Laidlaw's work^{1,2})

* Received for publication October 14, 1931.

Reducing Fluid: The developer is a mixture of strong neutral formalin (40 per cent formaldehyd) 1 cc., 1 per cent sodium carbonate in distilled water 3 cc., and distilled water to make 100 cc. Three minutes completes reduction.

Toning and Fixing: The toning bath is a 1:500 solution of Merck's "acid brown" gold chlorid in distilled water. The fixing fluid is the usual 5 per cent sodium thiosulphate ("hypo").

Variant 1

The sections are taken from distilled water, impregnated for 1 hour in the impregnating fluid, washed in 2 changes of distilled water and reduced in the developer for 3 minutes or so. They are then washed in tap water and toned for 3 or more minutes in the gold bath, washed, and fixed in the hypo for 3 or more minutes, after which they are washed and mounted in Canada balsam, after dehydrating in ascending percentages of alcohol and xylol.

Variant 2

This is similar to the preceding formula, except that the Laidlaw oxalic acid (5 per cent) bath is intercalated between the toning and fixing baths, and the fact that toning, redevelopment and fixing are all lengthened to 10 minutes each, to correspond with Laidlaw's directions.

*Variant 3**

In this variant formalin-soda replaces the oxalic acid procedure of its predecessor. It is made up exactly as before (formalin 1 cc., 1 per cent sodium carbonate 3 cc., distilled water to 100 cc.). Used developer should not be employed; it should be made up freshly each time. The treatment with the gold, formalin and hypo is the same as in Variant 2.

Variant 4

In the following three variants a tannic acid mordant is used made up as follows: pure tannic acid 0.2 gm., ammonium bromid 3.5 gm., strong neutral formalin 5 cc., distilled water to make 500 cc.

The sections are mordanted for 15 minutes in the tannic acid bath

* Instead of the soda-formalin solution a solution of 0.5 per cent oxalic acid in 5 per cent neutral formalin has been found to give better results and avoids the danger of precipitates. This was ascertained since the paper was submitted for publication.

formalin fixation gives more colorful results and is, on this account, to be preferred. This does not, however, imply that Zenker fixation is to be eschewed — quite the contrary; it gives very striking pictures in all instances and is well suited to the method.

Embedding: The ordinary routine method of paraffin embedding is used after dehydration of the tissue in ascending percentages of alcohol and in chloroform.

Preliminary Treatment: This is essential in the case of all the variants. The sections are deparaffinized in 2 changes of xylol and absolute alcohol and are then treated from 1 to 24 hours with a mixture of 2 parts pure pyridin to 1 part of pure glycerol. This bath keeps well and may be used repeatedly for many weeks. The sections are transferred directly from this to 2 changes of 95 per cent alcohol, washed in tap water and placed in distilled water.

Impregnating Fluid: This is a simple silver diammino hydroxid solution, depending upon the Kubie and Davidson formula.³ It is used in all the variants, at full concentration in the first three, at half strength in the last three. To 10 cc. of 10.2 per cent silver nitrate solution in distilled water, strong ammonia is added dropwise until the resulting brown precipitate is just dissolved; 10 cc. of 3.2 per cent pure sodium hydroxid solution in distilled water is added and the reprecipitated silver hydroxid again just dissolved by the addition of a few more drops of ammonia. The solution is then made up to 100 cc. with distilled water that has been heated to about 50°C. Sections are impregnated in this in a closed staining box in the incubator at 37° C, or the paraffin oven at 55° C for 1 hour in the case of Variants 1, 2 and 3, and for 10 minutes in the half-strength solution (5 cc. silver nitrate, 5 cc. sodium hydroxid) in that of the other three variants.

Silver diammino carbonate may be used interchangeably with, and in the place of the hydroxid; it often gives superior results, particularly in those variants in which the tannate mordant is used. It is made up at full strength in all cases; 10 cc. of 10.2 per cent silver nitrate, strong ammonia drop by drop until the precipitate is dissolved, and 10 cc. of 3.1 per cent sodium carbonate in distilled water, instead of the hydroxid. There is no reprecipitation upon adding the carbonate, as the hydrogen ion concentration remains unchanged, and further ammonia is therefore unnecessary. The solution is used in exactly the same manner as the hydroxid.

heated to 50° C in the incubator or paraffin oven. They are then treated for $\frac{1}{2}$ to 1 minute with 100 cc. of distilled water to which has been added 3 to 5 drops of strong ammonia. This is the "stop" solution. They are then washed for about 2 minutes in distilled water. The impregnation with silver is complete at the end of 15 minutes instead of 1 hour, as in the preceding variants. After impregnation the sections are washed in distilled water, developed, toned and fixed as in Variant 1.

Variant 5

Proceeding as in the preceding variant, the method changes as soon as the toning bath is reached, to correspond with Variant 2, lengthening the time to 10 minutes and using the 5 per cent oxalic acid-gold developer in exactly the same manner.

Variant 6

This resembles Variant 5 in every particular save one, formalin-soda developer replaces the oxalic acid bath, as in Variant 3.

The formalin-oxalic acid intensifier may be used here, as in Variant 3.

SUMMARY OF STEPS IN THE VARIANTS

1. Neutral formalin or Zenker fixation.
2. Paraffin embedding.
3. Pyridin-glycerol pretreatment for 1 to 24 hours.
4. In Variants 4, 5 and 6; tannic acid mordant for 15 minutes, followed by "stop" solution of ammonia for 30 seconds.
5. (a) Variants 1, 2 and 3; impregnation in warm silver diamino hydroxid for 1 hour.
(b) Variants 4, 5 and 6; impregnation in this bath at half-strength for 10 minutes.
6. Reduction of silver in formalin-soda developer for 3 minutes.
7. Toning in 1:500 gold chlorid in Variants 1 and 4 for 3 minutes; other variants for 10 minutes.
8. Reduction of gold in Variants 2 and 5 with 5 per cent oxalic acid; Variants 3 and 6 with formalin-soda; in either case for 10 minutes.
9. Fixing in 5 per cent sodium thiosulphate in Variants 1 and 4 for 3 minutes; other variants for 10 minutes.

TABLE I

Color Variations in Tissues Stained for Silver by 6 Variants

Tissue	Variant 1	Variant 2	Variant 3	Variant 4	Variant 5	Variant 6
Nuclei	Brown	Magenta, slightly brownish	Dull magenta-brown	Brown or black	Black	Sharp brown, reddish or black
Epidermal cytoplasm	Slate brown to brown	Slate violet to magenta	Rose slate, brownish to magenta	Slate brown to fuscous	Violet to violet-brown	Slate blue to slate brown
Glandular cytoplasm	Slate brown	Slate brown to magenta	Magenta-gray	Pinkish gray to brown	Pinkish gray to violet	Violet brown
Erythrocytes	Brown	Magenta	Dark brown to black	Reddish brown	Violet-brown	Brown to seal brown
Collagenous fibers	Lilac to light magenta	Deep magenta to violet	Dull magenta	Pinkish red to magenta	Magenta to scarlet-magenta	Brick red
Reticular fibers	Black	Black	Dark magenta to black	Pinkish red to magenta	Magenta to scarlet-magenta	Brick red
Endoneurial fibers Meissner's nervus cells	Red to black	Magenta to black	Magenta to violet or black	Red to black	Magenta to black, finest often carmine	Brick red
Skeletal muscle fibers	Slate brown striae black	Magenta to dark red, striae red to brown	Slate pink, striae red to brown	Pinkish brown striae black	Violet, striae deep magenta	Violet to black, striae indistinct, too intense
Cardiac muscle fibers	Gray	Magenta-gray	Slate pink	Gray, striae blackish	Violet, striae magenta	Violet, striae magenta
Smooth muscle fibers	Gray	Magenta	Rose-gray	Pinkish to brownish gray	Violet	Slate violet
Myelin sheaths	Black	Black	Black	Pinkish red to magenta	Magenta to scarlet-magenta	Brick red
Nerve trunks	Pink to red	Magenta	Magenta	Brownish pink, epineurium darker	Magenta, epineurium darker	Brick red, epineurium grayish
Melanin	Black	Blue-black	Black	Black	Blue-black	Black

and reticulum exactly alike, magenta or reddish. On the whole, the second and fifth variants will be found to be the best for general use. If the fifth is found to give too intense impregnations, the fourth or the sixth may be substituted. If one desires delicate effects with little or no disturbing cytoplasmic background, then the first variant should be the choice; or the fourth, if a little more cytoplasmic detail, color variety and plasticity are desired. Those variants depending upon the tannic acid mordant will give more colorful pictures, those omitting its use will tend to be monochromatic.

The reader is left to choose the variant that best suits his particular purposes and tastes; we can safely claim that he will find one of them that will fit his needs. Variant 2 gives ideal reticulum impregnation: it is particularly fine in the case of the "Gitterfasern," or reticulum of the liver sinusoids, the lymphoid reticulum and the sheaths of muscles. Variant 5 is particularly suited to the demonstration of muscle striations. With several variants, particularly Nos. 2 and 5, the medulla of the suprarenal is most admirably set off and demonstrated, and the Hassal's corpuscles of the thymus very well brought out because of their metachromatic impregnation.

The four figures in the plate (Figs. 1 to 4) demonstrate the various features of as many typical variants. They were made under exactly similar conditions, except for differences in the time exposure that were contingent upon the density of the impregnation. Four fields, as nearly similar to one another as possible, were chosen in four sections cut from the same block of tissue from a nevus.

It might be well to make some explanation of the different steps used in the six variants. The pyridin-glycerol treatment was originally introduced to increase the definition of the fibrillary structures in nevi, as it was known to be excellent in the case of nerve fibrils and the smaller nevus fibrils were suspected of being such. Whether they are, or not, the pyridin is found to bring them out more clearly than if it is omitted, and to keep down troublesome precipitates. The glycerol was added because it had been noted that Kaiserling III tissue impregnated more colorfully than that fixed in pure formalin. It was found that glycerol did, indeed, increase the metachromasia of the impregnation.

The silver solution is made up equimolar (0.6 molar), which explains the fractions in the "10.2" and "3.2" solutions of silver nitrate and sodium hydroxid. Sodium carbonate is added to the formalin

Note: Thorough washes are indicated between all steps, distilled water being required until the sections have been reduced in Step 6; after that tap water is employed throughout.

DISCUSSION OF RESULTS OBTAINED

After studying many sections from the experimental series used in our work we ran through a set of eleven sections in each variant, the material being taken from ten different organs (heart, two sections of lung, thymus, spleen, liver, kidney, suprarenal, uterus, lymph node and brain) from an autopsy performed almost immediately post-mortem. The color effects of the variants were then tabulated in the appended table. The sixty-six slides of the autopsy series were all impregnated at the same time and therefore represent a standard result.

As will be seen, the intensity of detail progresses through the series up to the fifth variant where it is most marked, and falls off a trifle at the sixth. The use of oxalic acid after toning the sections in gold chlorid develops the partially reduced gold salts that have replaced the silver and thus, by further reduction, "doubles" the impregnation: as a result one sees intensified and predominant magentas and violets, which are "gold colors." The use of a stronger reducer (formalin-soda) changes the picture from a prevalent magenta to a brick red, enhances the nuclear detail, impregnates the cytoplasm less densely, but does not produce as precise a fiber impregnation as does the weaker reducing agent (oxalic acid). This is probably explained empirically by the well known proclivity oxalic acid possesses for reducing gold salts.

It will be noted that the nuclei are listed as being either brown or black in those variants using the tannic acid mordant; there is no transition, they are either the one or the other. This phenomenon doubtless has its significance, occurring as it does in nuclei of the same cell race and apparently similar properties, but just what this may be we do not know. At first it was thought to be an artefact, but this peculiarity has been regularly noted in tannate sections. By referring to the table one may readily gauge the relative merits and drawbacks of the different variants. If it is desired to bring out reticulum selectively, then one of the first three variants should be selected, for the last three are unsuitable as they impregnate collagen

and to make the impregnation suitable for general use on almost any tissue. The use of the term "variant" might have been omitted, but it was thought that this clarified the slight variations in the procedure, so we have used that expression in this paper. It is not recommended that the method be used for impregnating brain or spinal cord sections, as there is not enough contrast to make it valuable in that connection.

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DESCRIPTION OF PLATE

PLATE 38

All photomicrographs were taken at about 800 diameters magnification by Mr. Joseph B. Homan of our Department of Medical Art, with the assistance of the authors.

FIG. 1. A field from a pigmented mole, or nevus, impregnated by the first variant. The nevus cells and fibrils are rather pale, the reticulum somewhat darker.

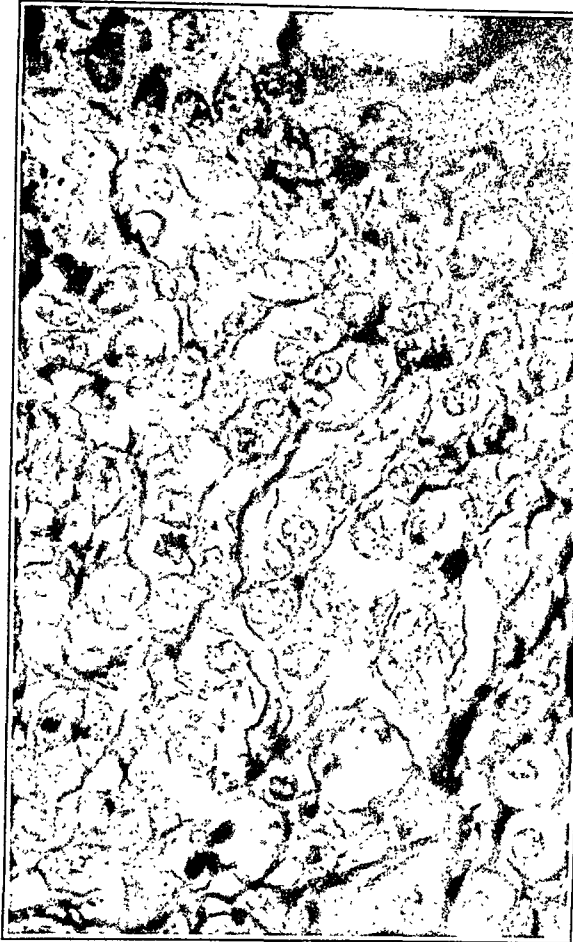
FIG. 2. Similar field impregnated by the second variant. The nevus fibrils are darker, the nuclear detail sharper.

FIG. 3. A third field impregnated by the fourth variant. Nuclei and fibrils still sharper. Note the occasional black nuclei.

FIG. 4. A field slightly deeper in the tumor, but otherwise identical with the preceding. Here the fifth variant was used. The excellent fibril and nuclear detail is at once apparent.



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Foot and Foot



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Silver Impregnation for General Purposes

thrombus had formed. In the group of cases reported from the Lahey Clinic and New England Deaconess Hospital,¹³ 1 case with thromboses in both auricles, which were attributed partly to coronary sclerosis, could be considered as developing in the same fashion; another showed evidence of a slight toxic myocarditis.

Two cases are reported here because they present in the myocardium chronic inflammatory changes and degeneration, without complicating factors, and definite enough to indicate the causal rôle of a toxin. The first patient died in thyroid crisis from unabated hyperthyroidism of the Basedow type: there was no operative or known iodine treatment. The second died in a reaction (postoperative storm) following bilateral thyroidectomy: a course of iodine treatment had been given.

CASE REPORTS

CASE 1. *Clinical History:* A female patient, 48 years of age, had suffered from struma, palpitation of the heart, exophthalmus, nervousness, and loss of weight for eleven years. Onset of illness followed the death of her father.

Physical examination showed a medium sized woman, incoherent and restless, with nutrition markedly reduced. Exophthalmus present. Tongue damp, cracked and tremulous. Thyroid moderately enlarged, lateral lobes easily palpable. Neck veins prominent. Lungs edematous at bases. Heart action visible, palpable at apex. Palpable pulsation of right ventricle. Heart enlarged by percussion; apex in the seventh intercostal space, three fingers' breadth from mammary line. Heart sounds obscured by indefinite murmurs. Blood pressure 125/75.

Treatment by several courses of strophanthin, glucose solution intravenously, sedatives and rest produced more regular heart action and relieved the dyspnea and delirium. Two weeks later vomiting, looseness of bowel movements, irregular heart action, pleural effusion and edema indicated the bodily collapse of thyroid crisis. The spinal fluid, blood leucocytes, blood sugar and non-protein nitrogen were normal. Blood culture was negative. After two days of marked irregularity and fibrillation of the heart, death occurred in the sixth week following admission.

Anatomical Diagnoses: Struma parenchymatosa (moderate); dilatation of both ventricles of the heart; chronic myocarditis with subendocardial hemorrhage; infarct of spleen (recent); congestion of lungs, liver, spleen, kidneys; edema of lungs; hydrothorax, right; atelectasis of right lung; chronic bronchitis.

Heart: Weight 475 gm. The left ventricle is considerably enlarged, its apex rounded; right ventricle and auricle appear widened. The epicardium is soft, smooth and shining. The foramen ovale

THE QUESTION OF A SPECIFIC MYOCARDIAL LESION IN HYPERTHYROIDISM (BASEDOW'S DISEASE)*

WILLIAM LEWIS, M.D.

(From the Pathological Institute of the Allgemeines Krankenhaus Eppendorf, Hamburg)

Reports in the literature are not consistent in describing morphological changes in the heart specifically related to hyperthyroidism. This variability can be attributed in part to the types of cases examined, to geographical situation, to method and duration of treatment, and to thoroughness of pathological examination. Fahr¹ in 1916 reported in 7 cases (5 of Basedow struma and 2 of colloid struma) degeneration and chronic inflammatory changes in the myocardium; again in 1921² he reported similar changes in a total of 27 cases (18 of Basedow struma, and 9 of colloid struma). These cases were both operative and non-operative. Goodpasture³ in 1921 reported fairly large foci of acute necrosis in the myocardium of 2 non-operative cases of hyperthyroidism with auricular fibrillation, in which "the cause of death was myocardial exhaustion." Goodall and Rogers⁴ in 1927 reported in 9 necropsies of Basedow's disease perivascular and interstitial polymorphonuclear infiltration and patchy necrosis of the myocardium; the changes were present in both ventricles, yet in most of these cases the pericardium, endocardium, valves and myocardium had appeared normal on gross examination.

Other reports, Müller⁵ (3 cases), Simmonds⁶ (8 cases), Pettavel⁷ (4 cases, later 12 in all), Wegelin⁸ (13 cases from the Bern Institute, mostly reported by Matti⁹ and Pettavel), Wilson¹⁰ (21 cases), Means and Richardson¹¹ (12 cases), Thomas¹² (1 case), Lewis¹³ (12 cases), McEachern and Rake¹⁴ (27 cases), record a variable degree of hypertrophy and dilatation, fatty changes and slight fibrosis. More marked changes, if present, were ascribed to rheumatic heart disease, hypertension or arteriosclerosis. In this group there were no changes considered definitely the result of toxic damage. However, Wegelin found in 1 case necrosis in a papillary muscle on which a

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The basal metabolism on admission was 57 per cent; after treatment with Lugol's solution, 5 drops 3 times daily for twelve days, it was plus 54 per cent. Lugol's solution was discontinued, and bromural, 0.5 gm. daily, given for three weeks. On bilateral strumectomy, two portions of thyroid (the size of an apple and the size of a walnut) were removed. Following operation the pulse rose from 90 to 140. Marked cardiac irregularity, delirium, tremor and restlessness followed. Despite emergency measures the patient died on the third day.

Anatomical Diagnoses: Status of postoperative partial strumectomy; fatty infiltration of liver; nephrosis (fatty infiltration of renal tubular epithelium).

Heart: Weight 300 gm. Epicardium, endocardium, valves and coronary vessels show no gross abnormalities. Ventricles are well developed. Myocardium is lax and of dull brown color.

Microscopic Examination: Both ventricles have foci of degeneration of myocardial fibers, increase of fibrous tissue, and small round cell lymphocytic infiltration. Capillaries are dilated, partly congested. Small coronary vessels are normal. Fat stains show diffuse fatty infiltration of the muscle fibers.

Thyroid: At autopsy a small distorted portion of tissue was found. The operative specimen consisted of an apple- and a walnut-sized portion of reddish brown tissue, partly encapsulated and showing degenerative changes. Microscopically this specimen consists of many small and medium sized follicles with cuboidal epithelium, some large follicles with low cuboidal epithelium and abundant colloid. There is some papillary hyperplasia, but the stroma between follicles is slight. There are several foci of fibrosis and old hemorrhage. The diagnosis is endemic goiter with secondary follicular hyperplasia and involutional changes.

Other Organs: The kidneys show marked fatty infiltration of the tubular epithelium (nephrosis). There is fatty infiltration of the liver. The spleen is congested; pancreas negative. The aorta is smooth and elastic. No thymic enlargement is present.

DISCUSSION

The explanation of the degenerative and inflammatory myocardial changes involves the question not only of a circulating toxin, but of a toxin apparently acting specifically on the myocardium. Whether the symptomatic effects of hyperthyroidism represent an excessive secretion of normal product of the thyroid gland (hyperthyroidism),

is patent to the extent of admitting a pencil point. The endocardium is smooth and shining. The myocardium of the left ventricle is reddish brown, glazed, and shows small, dark red, hemorrhagic, sunken foci. Under the endocardium of the left ventricle, of the interventricular septum, and of the left papillary muscle are small hemorrhagic foci. The coronaries and valves are negative.

Microscopic Examination: The left ventricle has numerous foci of hemorrhage, mostly under the endocardium and involving the adjacent myocardium; the foci are marked in the papillary muscle. The left ventricle also shows diffuse and focal fibrosis of the myocardium, mostly perivascular, accompanied by diffuse, small, round cell lymphocytic infiltration. There is degeneration of muscle fibers in the foci of hemorrhage, with lymphocytic infiltration and diffuse fibrosis peripherally. These changes are more marked at the distal portion of the small coronary vessels which show congestion and sometimes perivascular hemorrhage. Fat stains reveal diffuse fatty infiltration of muscle fibers, more abundant beneath the endocardium and in the papillary muscle. The larger branches of the coronary arteries are negative.

Sections of the right ventricle reveal moderate diffuse fibrosis, scattered lymphocytic infiltration, and some fragmentation and degeneration of myocardial fibers.

Thyroid: Each lateral lobe is firm and enlarged to the size of a small peach. Microscopically there are numerous follicles, low cuboidal epithelium, abundant colloid, fibrous interstitial bands, and some lymphocytic infiltration. The irregular size of the follicles, degree of involution of the epithelium (spontaneous — no known iodine treatment), and the fairly marked strumitis indicate a hyperplastic process of fairly long duration.

Other Organs: The aorta is smooth and elastic. No thymic enlargement is present.

CASE 2. Clinical History: A female patient, 50 years of age, had suffered from enlargement of the thyroid for twenty-nine years. No symptoms accompanied the onset of enlargement; later, there developed increase in size after menstruation. For ten years, especially in past year, there had been palpitation of the heart, headaches, tremor of hands, weakness on exertion, nervousness, excitability, sweating, attacks of diarrhea, and loss of weight. The physical findings accorded with the above symptoms. There was marked undernourishment. There was irregular enlargement in the region of the thyroid. The heart action was rapid, forceful, and irregular; signs of moderate cardiac hypertrophy were present.

blood stream; furthermore that this effect does not always occur, but when present is a condition of a true "Kropfherz" or goiter heart. Another opinion, of a theoretical nature, is that the increased metabolic processes and increased work demanded from the heart result in the myocardial damage. Or further, a view to which Goodpasture was inclined, hearts overstimulated by thyroid and laboring in a condition bordering on exhaustion are more susceptible to injury by toxic substances, *e.g.* from a mild terminal infection. This view, which has some experimental proof, does not preclude the possibility of a toxin of hyperthyroidism. Fahr found a "Kropfherz" in patients not only dying suddenly after operation and without evidence of terminal infection, but also dying with and without thymic enlargement. Furthermore, in some fatal cases, despite a terminal infection, the myocardium may be found normal at necropsy; in the hearts of four patients of one series,¹² no myocardial damage was evident, though bronchopneumonia had been a prominent factor in death.

In this question a comparison may be drawn with infectious disease. In diphtheria, for example, a toxin is always present; yet only in some instances may it act on the heart and kidneys to the extent that its effect is directly demonstrable or accompanied by anatomical changes.

SUMMARY

In some cases of hyperthyroidism, certain degenerative and inflammatory changes that occur in the myocardium indicate a toxic origin and suggest the presence of a toxin circulating in the blood stream. Two cases showing such myocardial changes are reported.

or the secretion of an abnormal and presumably toxic product (dysthyroidism) has not been settled. The view has been generally accepted by clinical investigators that the rôle played by the thyroid is one of hypersecretion. Hyperplasia of the gland is present, therefore hypersecretion. Removal of a portion of the gland, or irradiation, reduces the degree of activity. Involution of the hyperplastic epithelium (through the effect of iodine) accompanies, or is accompanied by, a moderation of symptoms. The ingestion of thyroxine or of dried thyroid gland produces symptoms in man and animals of thyroid overactivity.

On feeding thyroid material to animals, thereby inducing a toxic condition, changes in the myocardium have been observed. Bircher¹⁵ produced goiter in rats by feeding water from goitrous regions. The hearts showed macroscopic hypertrophy, microscopic cloudy swelling and fatty infiltration of muscle fibers, leucocytic infiltration and fibrosis. Farrant¹⁶ found in the hearts of cats and rabbits (fed thyroid tissue), general wasting and hyaline degeneration of fibers, with few nuclei and no transverse striations present. The animals showed a marked general toxic reaction, manifested by rapid loss of weight, bodily weakness and diarrhea. Death of the rabbits occurred after five to nineteen days. In white rats fed thyroxine, Hashimoto¹⁷ found focal myocarditis, later replaced by fibroblasts; these foci resembled Aschoff's rheumatic nodules. Takane¹⁸ reported leucocytic infiltration in the myocardium of his animals fed thyroidine and iodine salt. Goodpasture¹⁹ gave one group of rabbits thyroid gland and thyroxine; the animals showed characteristic clinical symptoms with definite, but relatively slight, myocardial lesions at autopsy. To a second group, similarly treated, he gave chloroform and found more striking, widespread myocardial necrosis. On the other hand, Rake and McEachern²⁰ recently failed to obtain any significant or specific changes in the myocardium of guinea pigs and rabbits "made hyperthyroid" by intramuscular administration of thyroxine. Other investigations, conducted chiefly for the effect of thyroid feeding on the size of the heart, report some degree of cardiac hypertrophy — Iscovesco²¹ in rabbits, Hoskins²² and Hewitt²³ in rats, Cameron and Carmichael²⁴ in rats and rabbits, Simonds and Brandes²⁵ in dogs.

Fahr concludes from his observations that the type of anatomical change in the heart indicates the action of a toxin circulating in the

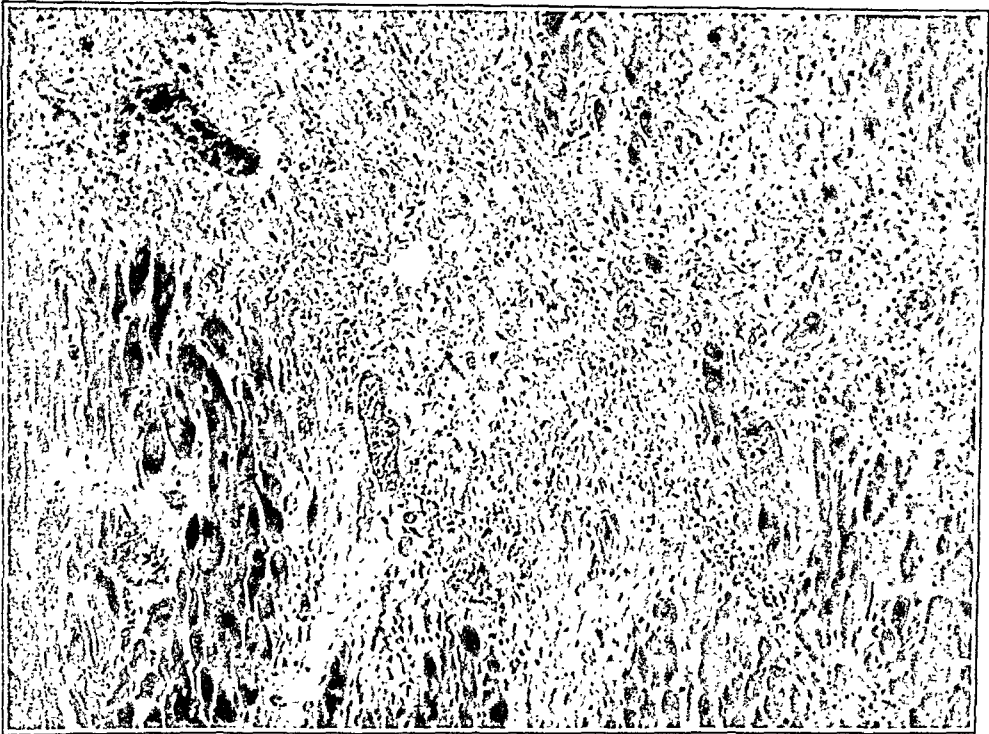
DESCRIPTION OF PLATE

PLATE 39

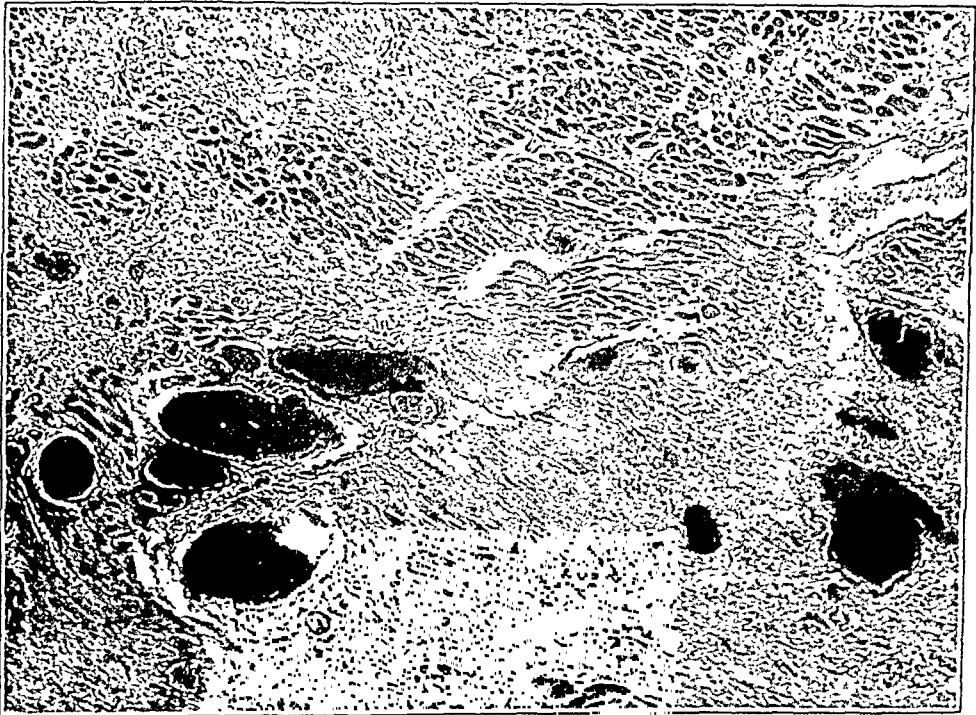
- FIG. 1. Case 1. Left ventricle. Focus of degeneration of myocardium; diffuse hemorrhage; diffuse lymphocytic infiltration; beginning fibrosis.
- FIG. 2. Case 1. Left ventricle. Degeneration of myocardium; diffuse hemorrhage; diffuse lymphocytic infiltration; congestion of capillaries.

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Different theories were advanced from time to time to explain the origin of the cysts in the generalized form of the disease, as well as the single solitary cysts. Beneke suggested that trauma was the basis for the formation of the solitary bone cysts and compared them to the apoplectic cysts of the brain. The correctness of this view was proved by my preceptor Pommer, in 1919, through the study of a case of cysts of the humerus in a 22 year old woman. From the study of the cyst contents and of the tissue surrounding the cyst Pommer established the direct proof for the hematomatous character of the process. The contents of the cyst, the hemosiderin deposits in its wall and in the bone marrow spaces of the surrounding bone, and furthermore the calcification of the fibrin deposits permit of no other explanation. The serous and albuminous contents of the solitary cysts are a mixture of the partly resorbed hemorrhages and new transudates. The pressure of hemorrhages upon the veins produces a stasis of tissue fluids, which causes the liquefaction of the blood content of many of the cysts, and edema of the tissues in the immediate vicinity. The blood vessels enclosed in the rigid compact bone have only a limited possibility of relieving disturbances of circulation, in contrast to the vessels in more loosely constructed organs.

The localized areas of loose fibrous tissue replacing the bone marrow were regarded by Pommer as effects of the congestion and irritation produced by the hemorrhages. These changes, designated as "phlegmasia," the result of a combination of congestion and reactive, irritative and inflammatory processes, are therefore secondary. As a result of functional mechanical factors and decreased fluid pressure a richly vascular and delicate network of new bone is often formed. This new bone is not a primary change, but, like the phlegmasia, a secondary result. The more active resorption of the surrounding bone is also a secondary result; it is dependent upon the pressure of hemorrhages and the increased transudation. These two factors, pressure from hemorrhages and increased transudation, produce increased bone resorption and eccentric atrophy of the bone and afford, also, the possibility for secondary hemorrhages with progressive cyst formation.

Following these fundamental observations by Pommer, Looser, in 1924, investigated the nature of the cysts in the so-called generalized osteitis fibrosa. Looser came to the conclusion that the multiple

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OSTEITIS FIBROSA *

F. J. LANG

(From the Department of Pathology of the University of Innsbruck, Innsbruck, Austria)

In 1891 von Recklinghausen described a complexity of changes in different parts of the bony skeleton. Such changes consisted in a transformation of the bone marrow into a fibrous connective tissue rich in giant cells, and a resorption of the compact cortex followed by a replacement with finely porous and often uncalcified new bone. Within these fibrous areas were cysts, which von Recklinghausen related to liquefaction — necrosis of the connective tissue. Here, also, he found the so-called solid brown tumors composed of numerous, multinuclear giant cells in a spindle cell matrix containing deposits of hemosiderin. These tumors were described as true giant cell sarcoma, or a tumor forming osteitis fibrosa, or as osteitis fibrosa with cysts and brown tumors.

In addition to this generalized osteitis fibrosa a localized type of the disease was described. Bones, otherwise uninvolved, contained localized areas of osteitis fibrosa with cysts and giant cells. These cases were accordingly treated as true sarcoma.

The discussion of the cause and origin of the cysts and giant cell tumors has produced a large and rich literature. Without exception until 1907 these brown or giant cell tumors were considered to be malignant. In this year Lubarsch, studying the case of Gaugele, brought forward proof that the giant cell tumor was an entirely benign formation of regenerative and resorptive character. Konjetzny was led to the same conclusion by his studies of the disease. Bloodgood considered the process a benign neoplasm and as early as 1910 advocated conservative treatment.

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it is possible to find the productive mechanism. This finding points to a relation between generalized osteitis fibrosa and osteomalacia. In the genesis of osteomalacia, disturbances of the calcium metabolism are responsible, and the parathyreoid gland is closely associated with such disturbances.

Such cases of osteitis fibrosa, associated with hyperplasia or tumors of the parathyreoid gland, do not contradict the conception which regards osteitis fibrosa as a secondary process dependent upon circulatory disturbances. In these cases the circulatory disturbances follow the pathological action of functional trauma upon the softened bone.

Regarding the relation between the parathyreoid gland and calcium metabolism, it is important to note that according to Biedl the hormone not only influences the secretion of calcium but also favors its resorption. As the calcium content of the blood rises, the elimination of calcium by the stomach decreases. The hormone possesses the property of fixing the calcium in the blood and closing its barriers of escape into the tissues. Hypersecretion by the parathyreoid gland leads to osteitis fibrosa, in which condition the blood calcium content is substantially increased. It is noteworthy that hyperplasia or tumors of the parathyreoid gland are frequently observed in osteomalacia and rickets.

The relation between parathyreoid hypersecretion and osteitis fibrosa is demonstrated by the recent researches of Jaffe, Bodansky and Blair. Through the administration of parathyreoid extract — for example parathormon — these investigators were able to influence calcium metabolism and produce osteitis fibrosa.

As already mentioned, Pommer traced the fibrous transformation of the bone marrow to localized congestion and irritative influences. Later, while studying a case of osteomalacia, he had a glimpse of the relation between osteitis fibrosa and osteomalacia. He was furthermore able to prove the dependence of osteitis fibrosa upon osteomalacia, and especially its dependence upon the mechanical effects of functional activity.

Following these observations, I made extensive studies (1925) to explain the genetic relation between osteomalacia, rickets and osteitis fibrosa. I found that the parts of the skeleton that are affected especially by mechanical function and strain undergo bending and cracking because of the insufficient calcification. Under

cysts of this disease also had their origin in the hemorrhages into bone marrow following trauma.

In spite of the great frequency of trauma in childhood, progressive cysts are found in relatively few cases. The site of the trauma is a decisive factor. Cyst formation does not follow injuries to the shaft of the bones as frequently as injuries to the epiphyses. The explanation lies in the fact that the richly vascular and porous juvenile epiphysis is especially prone to oft repeated hemorrhages subsequent to a comparatively slight trauma. It is also of great importance that the products of trauma — hemorrhage, edema and fluid — remain localized. Only in incomplete or complete fractures without tearing of the periosteum are the preliminary conditions present for the formation of cysts. Under these circumstances a primary hemorrhage leads to pressure upon the efferent vessels. Finally the maintenance of functional activity, either partial or total, acts as a pathological irritant, important for the production of secondary hemorrhages with the above mentioned sequellae. Whenever the trauma tears the periosteum, even in incomplete fractures, the blood escapes into the surrounding soft tissues and cyst formation does not follow.

The interpretation by Lubarsch of the so-called giant cell tumor as a regenerative and resorptive process, and the recognition by Pommer of the blood cysts as a progressive hematoma formation clarified the developmental picture of osteitis fibrosa. Thus the two processes, which were considered characteristic of the so-called generalized osteitis fibrosa, were shown to be dependent on localized conditions. Furthermore, after this knowledge of the origin of the "phlegmasia" changes in the bone marrow, there no longer remained the necessity of explaining the development of the loose, fibrous transformations of the bone marrow as an independent form of osteitis fibrosa.

Yet, almost everywhere, osteitis fibrosa was considered as an independent disease and its origin was ascribed to various causes. Inflammatory processes especially were considered responsible for the localized type of the disease. Other observers believed the localized as well as the generalized form of osteitis fibrosa to be a disease of unknown origin. Von Recklinghausen advanced the conception that generalized osteitis fibrosa was definitely related to osteomalacia. In some instances the osteofibrotic changes are associated with hyperplasia or tumors of the parathyreoid gland, and in these cases

osteitis fibrosa will be made, thus confirming the current misconceptions on this subject.

Of all the conditions that are responsible for the origin of osteitis fibrosa, the most decisive are the peculiar structure and the circulatory system of bone which allow only a slight possibility for compensatory adaption to circulatory disturbances. Furthermore, use of the bone acts as an irritant under the given pathological conditions.

The functional viewpoint has proved to be of the greatest importance in explaining the question of the relation between osteitis fibrosa, osteomalacia and rickets. Likewise in general pathology the application of the functional viewpoint promises to clarify the origin and relationship of many obscure pathological changes.

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these conditions, due to the peculiar structure and circulatory system of the bone, a permanent congestion of the blood and lymph vessels is produced. This congestion, together with the continuous mechanical irritation, leads to osteitis fibrosa. The fibrosis of the bone marrow and the increased rebuilding of bone are, therefore, not independent processes; they are secondary results of the localized circulatory disturbances which follow the action of functional, mechanical trauma upon insufficiently calcified and softened bone.

This year (1931) I confirmed these conclusions by experimental and clinical studies of rickets and scurvy, and by further studies of osteomalacia. In all these diseases I found regularly osteitis fibrosa (Figs. 1 to 8).

The development of osteitis fibrosa following a trauma, producing a congestion, a resorptive inflammation and finally a progressive hematoma, is comparable to the induration in parenchymatous organs following chronic passive congestion. Osteofibrotic changes are also observed in inflammatory processes as a result of the congestion. An example of this is to be found in odontogenetic osteitis fibrosa of the jaw. Similar changes exist in the immediate vicinity of metastases to bones, as Wagoner has pointed out, in callus formations and pseudo-arthritis, in gout, and about tubercular and syphilitic bone lesions. By these processes are produced the same pressure and congestion influences as occur after traumatic hemorrhage into the bone marrow.

This large and varied material establishes beyond doubt the secondary nature of osteitis fibrosa and disproves the theory that it is two independent processes.

To close with a few general considerations: von Recklinghausen advanced the opinion that, in the bony system, the secondary symptoms and sequellae control the disease picture and mask the basic factors. This important conception explains the great difficulty in arriving at a definite conclusion from the final picture of the disease process. We have seen that in rickets, scurvy and osteomalacia, the disease picture is dominated by osteitis fibrosa. It is only by exacting histological methods, designed to show both the calcified and non-calcified bone, and by the study of many large sections from various bones, seeking the primary changes, unobscured by osteitis fibrosa, that we were able to establish the basic nature of the disease. Without such careful methods, an incorrect diagnosis of primary

DESCRIPTION OF PLATES

PLATE 40

- FIG. 1. Osteitis fibrosa of the alveolar bone of the lower jaw of a dog in experimental rickets, with rebuilding of the bone and fibrosis of the bone marrow. Hyperemia of the blood vessels is shown.
- FIG. 2. Microscopic section of the humerus of a 2 year old girl with rickets.
- FIG. 3. Osteitis fibrosa in rickets, showing deeply stained (calcified) bone surrounded by a newly formed, non-calcified bone. Hyperemia of the fibrotic bone marrow is also shown. High power picture of Fig. 2.
- FIG. 4. Femur of a 3 year old girl with rickets, showing new formation of uncalcified bone and fibrosis of the bone marrow.

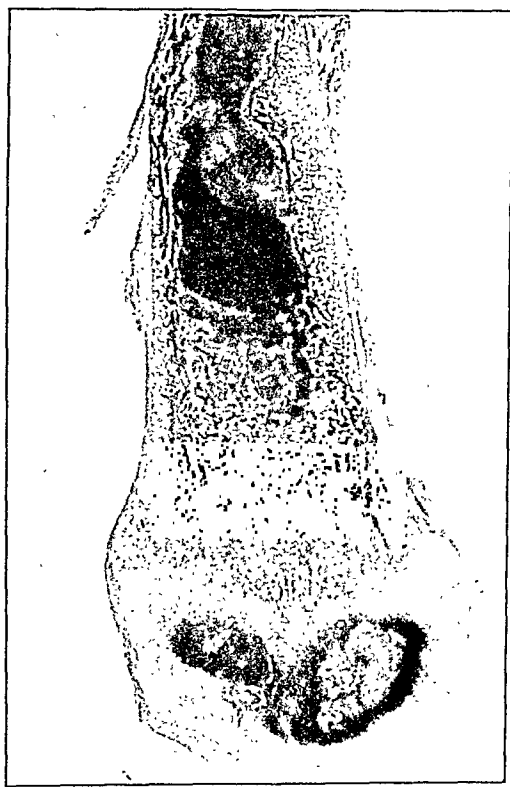
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PLATE 41

- FIG. 5. Fibrosis of the bone marrow and rebuilding of the bone in rickets in a 10 months' old boy. On the left side of the figure lymphoid bone marrow is seen.
- FIG. 6. Tibia, showing "phlegmasia" of the bone marrow and increased rebuilding of the bone in a case of osteomalacia in a 48 year old man.
- FIG. 7. Fibrosis of the bone marrow and new formation of uncalcified bone in osteomalacia in a 78 year old woman. Calcified bone deeply stained.
- FIG. 8. Osteitis fibrosa with fibrosis of the bone marrow and rebuilding of the bone in osteomalacia in an 80 year old woman.



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Lang

Osteitis Fibrosa



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Lang

Osteitis Fibrosa

Guarnieri bodies represent, in part at least, colonies or masses of Paschen corpuscles.

TECHNIQUE

Bacteria-free virus in fresh infected rabbit's testis (Levaditi neurovaccine) was used to initiate the infection. While we have been able to infect the chick membrane with glycerinated virus, the result is more uncertain, and we recommend the use of fresh infected tissue from the rabbit's testis as a constant source of original virus.

Since the infection "takes" rapidly and the lesion evolves readily, requiring only two or three days for its development, we have found it most satisfactory to use chick embryos of 12 days' incubation. At this stage the membrane is well formed and easily accessible. By candling the egg the air-cell and the membrane can be outlined with a wax pencil. A thin coat of melted paraffine is laid over the shell where the window is to be cut. The egg is then placed in a bowl of warm water (40° C) and rested upon a mass of plasticene which has been properly indented to receive it. This holds the egg in the suitable position. The water should come almost up to but not over the paraffined surface. With a hard steel trocar, ground with a triangular end to a sharp point, a window 1 or 1½ cm. square is cut in the paraffined surface. If the sides have been well cut the overlying shell can be easily removed by lifting it at one corner with fine pointed forceps. The exposed shell membrane may be unruptured, though frequently it is more or less injured. A thin coat of melted paraffine (about 45° C) is laid over the cut edges of the shell and the exposed shell membrane with a cotton swab. With the point of a pair of fine curved forceps the shell membrane may be torn from beneath outward on three sides, folded outward and the fourth side cut with small scissors. These procedures tend to prevent infection from broken bits of shell. The instruments used are kept sterile by passing them through the flame of a Bunsen burner.

When the membrane is exposed a bit of infected tissue (rabbit testis or membrane) about the size of a pin-head is placed upon it. A mixture of sterile vaseline and paraffine in a 10 cc. record syringe is used to lay a ring about the opening and upon this a sterile cover glass is placed and pushed down to seal it completely. The egg is then returned to the incubator with the window up. It may be ex-

VACCINAL INFECTION OF THE CHORIO-ALLANTOIC MEMBRANE OF THE CHICK EMBRYO *

E. W. GOODPASTURE, M.D., ALICE M. WOODRUFF, PH.D., AND G. J. BUDDINGH, A.B.

(From the Department of Pathology, Vanderbilt University Medical School,
Nashville, Tenn.)

Notwithstanding a certain amount of confusion which has at times existed concerning the possibility of an identity of fowl-pox and variola-vaccinia, there are now several studies on record which show that although chickens are susceptible to infection with vaccine, fowl-pox is a distinct disease both immunologically and in the cytology of its lesions (Levaditi and Nicolau, Ledingham, Lowenthal *et als.*, Andervont, Woodruff). Andervont¹ was able to demonstrate Guarnieri bodies in vaccinal lesions of chickens, and this was confirmed by Woodruff,² who pointed out essential distinctions between these inclusions and the Bollinger bodies of fowl-pox.

In a recent communication we reported the successful infection of the chorio-allantoic membrane of chick embryos with vaccinia virus, following the method of inoculation described by Woodruff and Goodpasture³ in their study of fowl-pox of this membrane.⁴ In the present report we wish to describe in greater detail vaccinal infection of the membranes of the chick embryo, with especial reference to the cytology of the lesion and the seeming relationship between the Guarnieri bodies and the Paschen corpuscles which constitute the specific elements.

While the Guarnieri bodies are by every investigator of the subject regarded as specific for variola-vaccinia, there is perhaps still skepticism as to the specificity of the Paschen granules and no one as yet has been able to demonstrate a relationship between these two important structures, although many agree with the view of Paschen that the elementary corpuscles represent the actual virus of the disease.⁵

Vaccinal lesions in the chorio-allantoic membrane of embryo chicks afford an unusual opportunity to study both of these morphological elements and, we believe, furnish good evidence that the

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Infected chick embryos usually die on the fourth day after inoculation, and we have found the 48 hour period best for transplantation and general study, though 72 hours is often a satisfactory interval.

SERIAL TRANSFERS

In one series of experiments the virus was carried through eight generations in embryos, in another series it reached the fourteenth generation. For some reason, probably technical, the fifteenth generation failed to "take." In these passages there did not appear to be any diminution in virulence, so far as the appearances of the lesions were concerned.

The virus in the eighth generation was inoculated upon the skin of baby chicks after plucking the down. Macroscopic nodules, about 1 mm. in diameter, corresponding to down follicles, appeared at the site of inoculation within three days. Remaining apparently stationary for two or three days they rapidly receded. Two weeks after their recovery from vaccinia these chicks were inoculated with fowl-pox virus, and infection ensued which ran a typical course. This experiment confirms the result of other observers, that vaccinia in the chick confers no immunity to fowl-pox.

HISTOLOGY

The infected membrane may be considerably thickened, though not so much so as in fowl-pox. The swelling is due to a variety of causes, least of all to cellular hyperplasia, which is the chief response to fowl-pox.

In the vaccinal infection inflammatory changes and hemorrhage are mainly responsible for the increase in thickness, and this is especially marked in the older areas of involvement. At the extreme advancing edge one finds the latest effects which are characterized by moderate edema, perhaps some capillary hemorrhage and slight hyperplasia, both of ectodermal epithelium and endothelial cells. The entodermal epithelium is slightly, if at all, affected. As the earlier infected areas are approached, ectodermal epithelium is found to be necrotic and its capillaries filled with cellular debris. Inflammatory exudate in the mesodermal layer is increased in abundance and consists largely, in addition to red blood cells, of polymorphonuclear leucocytes. There are also admixed with

amed on succeeding days by placing the window under a dissecting microscope.

When it is desired to open the egg to examine and remove the infected area, the cover slip is pulled off and frequently the vaseline comes off with it. If not, it can easily be scraped off with a sterile scalpel. The window is enlarged to the desired size by breaking off the edges with sterile forceps. The infected membrane may then be cut out with small scissors and placed in a petri dish containing sterile isotonic fluid.

THE VACCINAL LESIONS OF THE MEMBRANE

After 24 hours the membrane appears somewhat thicker, grayer and more opaque about the bit of inoculum, and after 48 hours there is a zone about 1 cm. wide, thickened, gray, opaque and usually flecked with small hemorrhages. Sometimes almost the entire area is red and hemorrhagic. At this stage there is a gray advancing margin which is best for histological study. In the center there may be a brownish area of necrosis, variable in size. After the lesion has been extirpated a bit of tissue from the thickened and hemorrhagic area is removed with scissors and smears from this are stained by Morosow's method ⁶ to determine the presence of Paschen bodies. These bodies appear in enormous numbers in the infected membranes at the 48 hour period and after. Their presence is diagnostic of vaccinal infection. Other smears are stained with Loeffler's methylene blue to determine the presence of bacteria. If Paschen corpuscles are abundant and no bacteria are demonstrable, a piece of infected membrane about 0.5 mm. in diameter is inoculated upon the membrane of each of five or six embryos for continuing the passage. Pieces of the remaining infected membrane are then inoculated into culture media and others are kept in the icebox or in glycerol (50 per cent in 0.9 per cent saline). Tissue for histological study is taken from the advancing margin and fixed immediately.

The preparations used in the present histological study were fixed in Zenker's solution (10 per cent glacial acetic acid), and stained in a 2 per cent aqueous solution of acid fuchsin for 10 to 30 minutes, washed and counterstained about 30 seconds with Loeffler's methylene blue, differentiated in absolute alcohol, cleared in xylol and mounted in cedar oil.

material is very abundant and sometimes almost completely replaces the cellular cytoplasm. In other cells it is more dispersed and is scattered through the cytoplasm in fine and coarse granules. Similar intracytoplasmic masses, often reaching a relatively large size, are found abundantly in endothelial cells and fibroblasts of the mesodermal layer. These masses have the morphology and the staining characteristics of Guarnieri bodies. They are especially well marked in the cells composing the endothelial nodules. Isolated fibroblasts show them quite distinctly. They are to be found typically also in endothelial cells lining veins and capillaries. Adventitial cells, as well as endothelial cells, composing the walls of larger blood vessels, often show these characteristic Guarnieri bodies, and one sometimes gets the impression that they are present in smooth muscle cells as well, but this is still doubtful.

The entodermal cells lining the infected area frequently contain numerous Guarnieri bodies, and here they have more the typical structure of these inclusions as they are usually to be seen in the corneal epithelium of the infected rabbit's eye. That is to say, they are apt to be small, single, discrete, more compact and more densely stained. They lie in a clear space next to the nucleus. In areas of hyperplasia of entodermal cells, however, they may become larger and more granular, simulating those of the ectodermal and mesodermal cells.

One gets the impression that the entodermal cells offer considerably more resistance to the infection than the cells of the other two germinal layers, and this may account for the variation in the morphology of the Guarnieri bodies.

It is to be emphasized that the chief, if not all "included," material within any of these cells is that which composes the Guarnieri bodies. This is of great importance in view of the appearance found in smears from the membranes stained by Morosow's method to demonstrate Paschen corpuscles.

PASCHEN CORPUSCLES

If fragments of fresh, infected membrane be placed in distilled water and examined under the oil immersion lens, one frequently sees round or oval masses, apparently within the cytoplasm of cells, which are composed almost entirely of minute, uniform granules oscillating rapidly in Brownian motion. These granules have the

these, large rounded mononuclear cells which may be either dis-oriented endothelium or fibroblasts. Occasionally mitotic figures are found in the large cells. The entodermal epithelium in these areas may show considerable hyperplasia, but usually no necrosis. In the earliest areas of infection these changes are accentuated, and there may be inflammatory cells in the entodermal layer and necrosis. At the advancing margin, capillary endothelium sometimes undergoes active focal hyperplasia, indicated by small isolated groups and whorls of these cells in the form of tiny nodules.

From a histological standpoint the virus seems to affect ectodermal epithelium first and most profoundly, mesodermal cells less markedly and entodermal epithelium least of all. No vesicles are formed. The infection seems to spread diffusely and centrifugally, affecting the entire membrane as it goes. There is no evidence thus far that lesions occur in the embryonic tissues other than at and about the site of inoculation in the chorio-allantoic membrane. We have not yet made a study of the dissemination of the virus in the embryo.

GUARNIERI BODIES

In sections stained to demonstrate Guarnieri bodies, very marked changes are to be found in practically all types of cells of the fixed tissue. In order to study these changes to the best advantage one must examine the latest stages in the advancing edge of the infection in the membrane. Injury is profound and the cells rapidly undergo disintegration. It seems probable that there is only a short interval in which the cellular inclusions may be seen to best advantage. The Guarnieri bodies develop in abundance in the cells before there is any evidence of inflammatory cellular exudate.

Guarnieri bodies are best observed in the ectodermal cells. Practically all of these cells in the recently infected areas, when intact, show the "included" material in their cytoplasm. Here the bodies occur in irregular clumps and masses, perinuclear or paranuclear in arrangement. The cytoplasmic masses are granular and rather amorphous. The material stains for the most part with fuchsin in our preparations, though sometimes it is flecked with bluish granules. The granular material is fairly dense when properly differentiated and lies in a clear area. The inclusions, when single, are often triangular in shape with the base next to the nucleus. The Guarnieri

so that in smearing they do not rupture to spread their content of corpuscles. The action of trypsin has the opposite effect, in that it tends to disassociate cells and to rupture them; consequently dispersed Paschen corpuscles are extremely abundant in the smears from tissue treated with it.

DISCUSSION

It has recently been shown that the specific cellular inclusion (Bollinger body) of fowl-pox is composed in large part of uniform granules which correspond in their morphology, numbers and staining reaction with the Borrell bodies previously demonstrated in smear preparations. Furthermore, these specific inclusions have been isolated, washed and inoculated, both whole and in fractions, into chickens, with resulting successful infections.⁷ Thus it has been shown that the cellular inclusion of fowl-pox carries the infectious agent; and the evidence is very strong that this agent is morphologically represented by the Borrell granules.

In molluscum contagiosum the specific inclusions have also been shown to be composed of minute granules (Lipschütz granules) which correspond in size, numbers and staining reaction with the Borrell granules of fowl-pox. Owing to the sticky consistency of these intracellular masses it has not been possible to isolate them and prove their infectiveness, although in all other respects, including resistance to tryptic digestion, the Lipschütz granules resemble the Borrell corpuscles.⁸

In these two viral infections, characterized clinically by a pox, it has been determined that the specific cellular inclusions are composed in large part of uniform corpuscles which are small enough to be filterable and numerous enough to account for the infectiousness of great dilutions of original material.

Variola-vaccinia is also characterized by a pox, and there are specific cellular inclusions (Guarnieri bodies) in the lesions. Furthermore, Paschen and others have demonstrated the great constancy of minute corpuscles (Paschen corpuscles) in smears from early lesions. These granules have the same size and staining qualities as those of Borrell and Lipschütz, and they have been shown to be agglutinable by vaccinal immune serum (Paschen, Ledingham⁹). Up to the present time, however, it has not been possible to determine any relation between the Guarnieri bodies and the Paschen

size, uniformity of structure and numbers which correspond to the Paschen bodies so abundantly demonstrable in smear preparations. The masses are not so numerous, however, as Guarnieri bodies are known to be in the same tissue, and we have not been able positively to correlate the two structures in fresh preparations.

Smears made directly from a piece of fresh, untreated membrane and stained by the Morosow method show enormous numbers of Paschen corpuscles diffusely scattered, and also in distinct masses.⁶ It appears from these smears that the Paschen bodies occur originally in rather sticky groups which, in the process of making the smear, adhere to each other, forming relatively large clumps of material resembling closely similar masses occurring in smears from fowl-pox. About the edges of these clumps are the discrete Paschen bodies which give the clue to the composition of the whole.

In the smears made directly in this way there are a very few intact cells, so that it is difficult usually to detect any relationship between the Paschen corpuscles and cells. However, if a piece of membrane is placed in 1 per cent trypsin solution for 30 minutes, it becomes softened and smears made with it show many intact cells that evidently have become dislodged from the loosened stroma. Many of these cells show in their cytoplasm one or more masses corresponding in size to the Guarnieri bodies, and in those cells which are spread out or partially ruptured by smearing, it can be seen readily that the intracellular structures are composed of compact agglomerations of uniform, round, minute corpuscles, measuring approximately 0.25 microns in diameter, although enlarged by the staining method. These are the Paschen corpuscles, and from a morphological standpoint we have no doubt that the intracellular masses of them are identical with the Guarnieri bodies found so abundantly and so large in the stained sections. No other observed intracellular structures could account for them.

Another experiment indicates that all the dispersed Paschen corpuscles originate from ruptured cells. If one places a small piece of membrane in 1 per cent acetic acid for 5 minutes, then washes it and makes a smear, there are few if any dispersed Paschen corpuscles, but hyaline masses, for the most part compact, are present within and about the cells; and some of these are to be found smeared out thin enough to see that they are composed of the minute Paschen bodies. The acetic acid evidently fixes the cells, at least partially,

luscum contagiosum, restricted in its growth to ectodermal epithelium, through fowl-pox, which will grow apparently only in ectodermal and entodermal epithelium, to vaccinia, which finds its growth requirements satisfied in cells of all three germinal layers.

This fact no doubt has a bearing upon the cultivability of these viruses in tissue culture. Molluscum contagiosum would probably require a culture of ectodermal epithelium to initiate its growth. We have not succeeded in cultivating fowl-pox in cultures of mesodermal cells of the chick, and the cytology of its lesions suggests that it needs either a culture of ectodermal or of entodermal epithelium. Many investigators, however, have been able to cultivate vaccinia virus through several generations in a medium containing mesodermal cells of the chick, and our investigations confirm, from a cytological standpoint, the possibility of a generation of vaccine in either endothelium or fibroblasts.¹²

The method of infecting the chorio-allantoic membrane of chick embryos with vaccine offers an opportunity for further studies upon the Paschen corpuscles which appear in the lesions in great numbers, and it also provides a means of generating large quantities of sterile vaccinal virus.

SUMMARY

1. Vaccinal lesions of the chorio-allantoic membrane of chick embryos are described.
2. Guarnieri bodies have been demonstrated for the first time in mesodermal cells (endothelium and fibroblasts).
3. Evidence has been presented that the Guarnieri bodies are composed in part of Paschen corpuscles.
4. Similarities between Borrell corpuscles (fowl-pox), Lipschütz corpuscles (molluscum contagiosum) and Paschen corpuscles (vaccinia) are pointed out.
5. It is suggested, on the basis of the cytology of the lesions, that the virus of molluscum contagiosum would require a culture of ectodermal epithelium to initiate its growth outside the body; fowl-pox would require either ectodermal or entodermal epithelium; while vaccinia would multiply in a culture of cells from any of the three germinal layers.

corpuscles, although Ewing¹⁰ was able to demonstrate in Klatsch preparations a granular composition of some of these structures in cells from the rabbit's cornea. By analogy with fowl-pox and molluscum contagiosum one would suspect that the former might be in part composed of the latter. Our experiments seem to show that this is the case.

Observations which support this conclusion depend upon the fact that vaccinal infection of the chorio-allantoic membrane of chick embryos results in the appearance of unusually abundant and large Guarnieri bodies, and in extremely numerous Paschen bodies. In smear preparations from these lesions it has been possible to demonstrate structures which are interpreted to be Guarnieri bodies partially disintegrated within the cells, so that their component granules can be seen readily.

Although Paschen has frequently observed the corpuscles of vaccinia inside cells in smear preparations, he has not been able to relate them definitely to the Guarnieri body. He interprets them, however, to be the infectious agent and judges that they multiply in part, at least, inside the cells.

There is thus strong evidence, not only that the viruses of vaccinia, fowl-pox and molluscum contagiosum are cytotropic in the usual sense of having an especial affinity for cells, but that they are *cytotrophic*, if we may use this term to mean that they require under natural conditions an intracellular environment for their growth.

There is an interesting variation in the cellular affinity of the three viruses under consideration, brought out in the case of two of them by infection in the chorio-allantoic membrane of the chick embryo. Molluscum contagiosum has not yet been successfully engrafted upon a host other than man, but so far as one knows, it affects only and specifically ectodermal epithelial cells in this natural host.

Fowl-pox, readily inoculable upon the chick membrane, affects both ectodermal and entodermal epithelium, although the latter is changed relatively slightly. Vaccinia, on the other hand, affects cells of the three germinal layers, ectoderm, mesoderm and entoderm. In our studies it has been shown for the first time that Guarnieri bodies may occur not only in ectodermal and entodermal epithelium,¹¹ but also in endothelial cells and fibroblasts. There is thus an increasing latitude of cellular affinity as we pass from mol-

DESCRIPTION OF PLATES

All photomicrographs taken at a magnification of 1800 diameters.

PLATE 42

- FIG. 1. Epithelial cells of ectoderm showing large irregular Guarnieri bodies.
- FIG. 2. Epithelial cells of entoderm showing more typical Guarnieri bodies.
- FIG. 3. Epithelial cells of ectoderm showing irregular Guarnieri bodies diffusely distributed through the cytoplasm.

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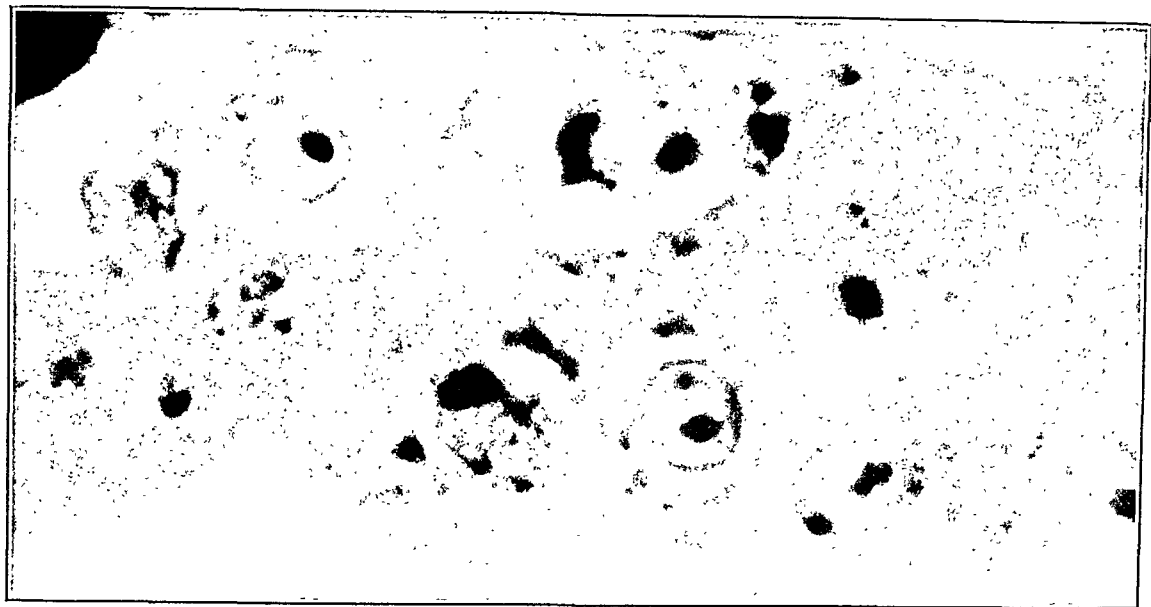
PLATE 43

FIG. 4. Vein showing two Guarnieri bodies within lining endothelial cells.

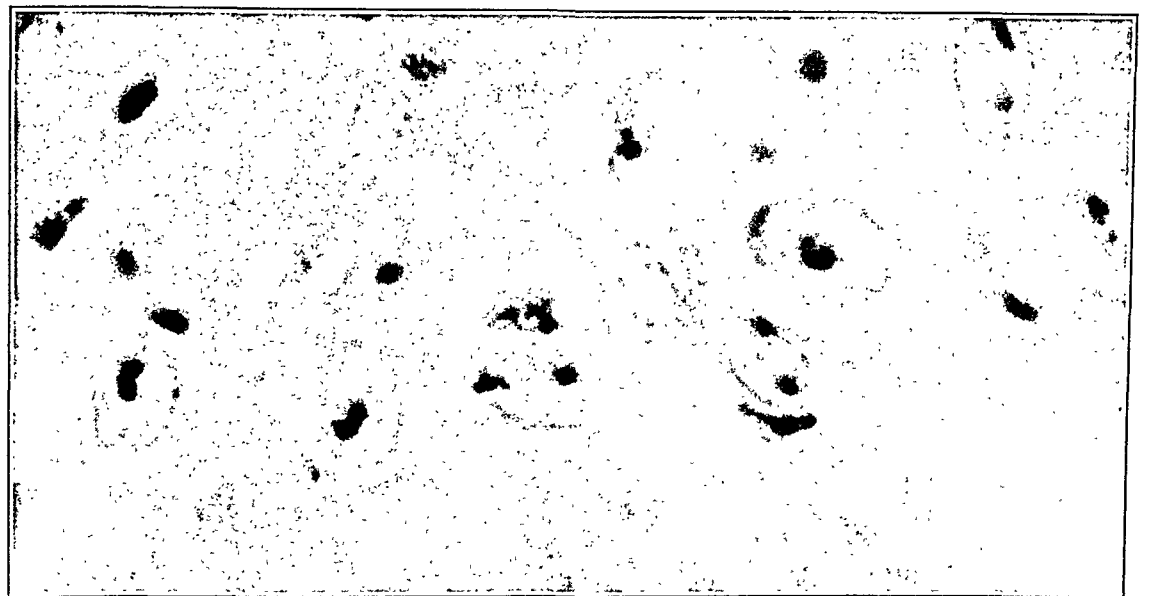
FIGS. 5 and 6. Guarnieri bodies in capillary endothelium.

FIGS. 7 and 8. Guarnieri bodies in fibroblasts.

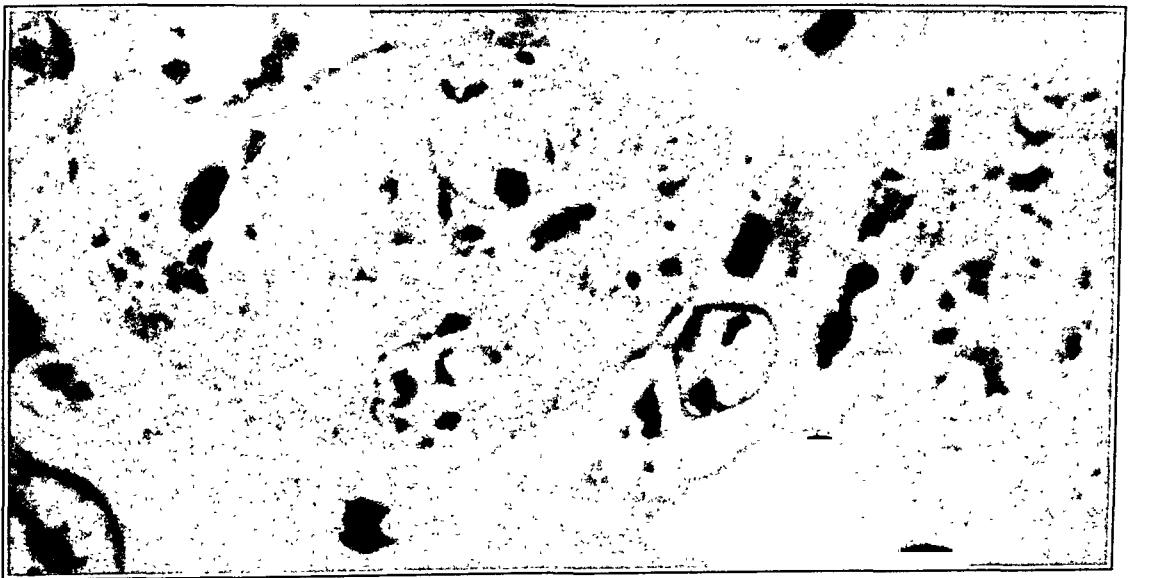
FIG. 9. Endothelial "nodule" showing Guarnieri bodies.



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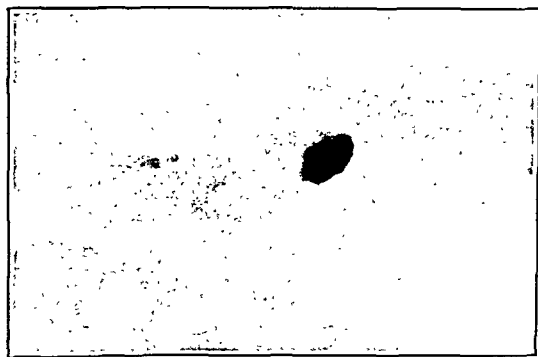
PLATE 44

FIG. 10. Endothelial "nodule" showing Guarnieri bodies.

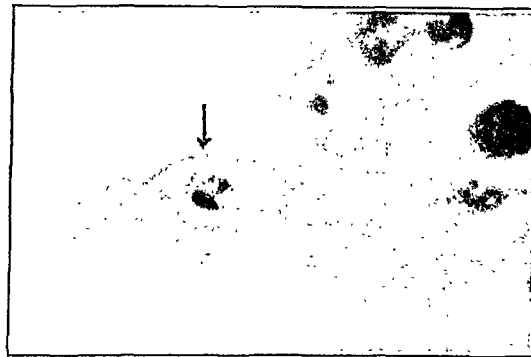
FIGS. 11 and 12. Cells in smear preparations showing unresolved intracytoplasmic masses, which in thinner portions are seen to be composed of Paschen corpuscles.



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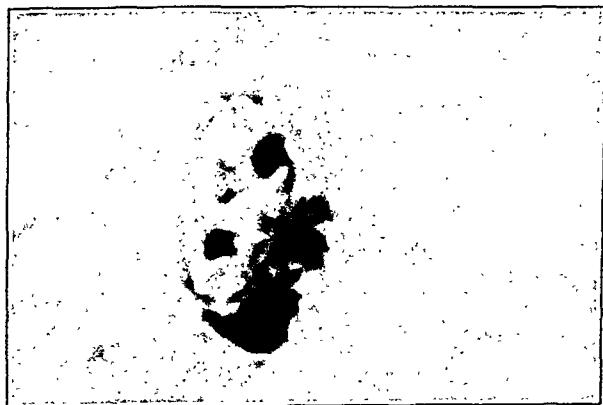
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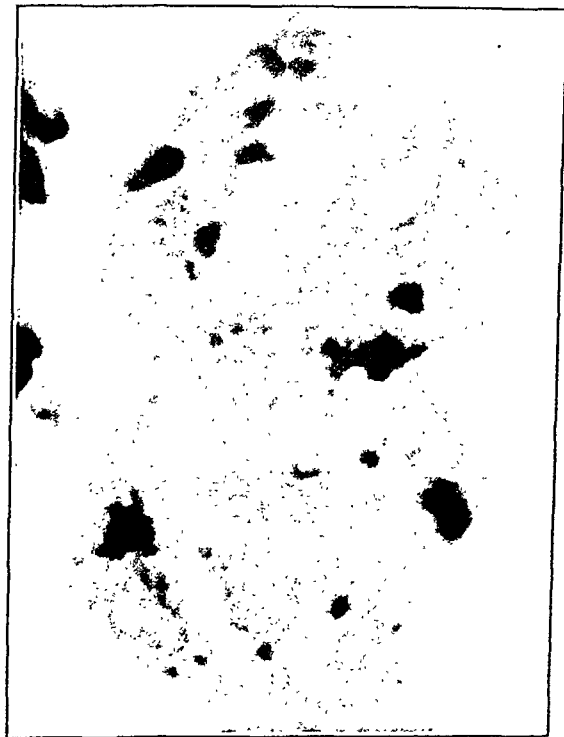
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PLATE 45

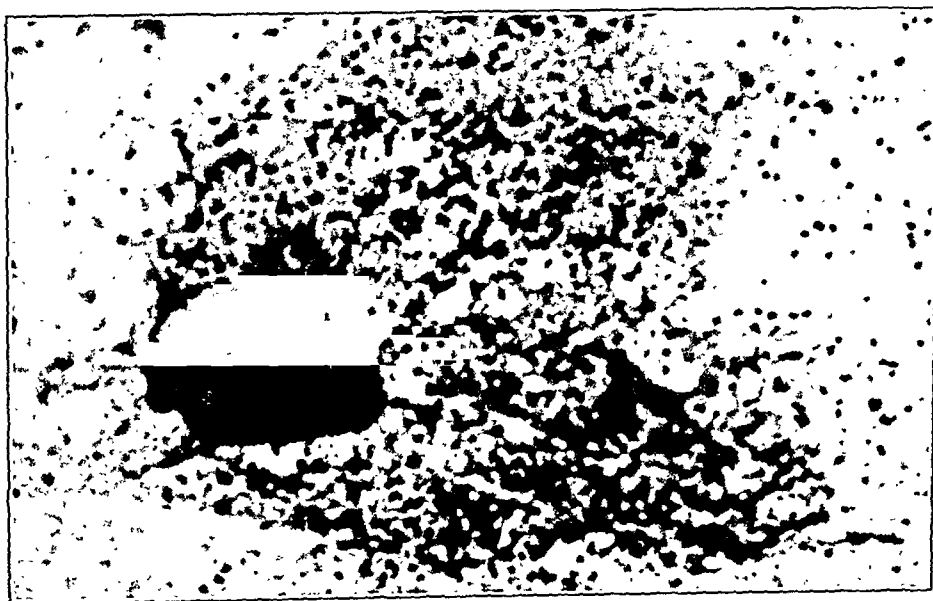
FIGS. 13 and 14. Cells from a smear preparation showing unresolved and resolved intracellular groups and masses of Paschen corpuscles. K₂ filter.



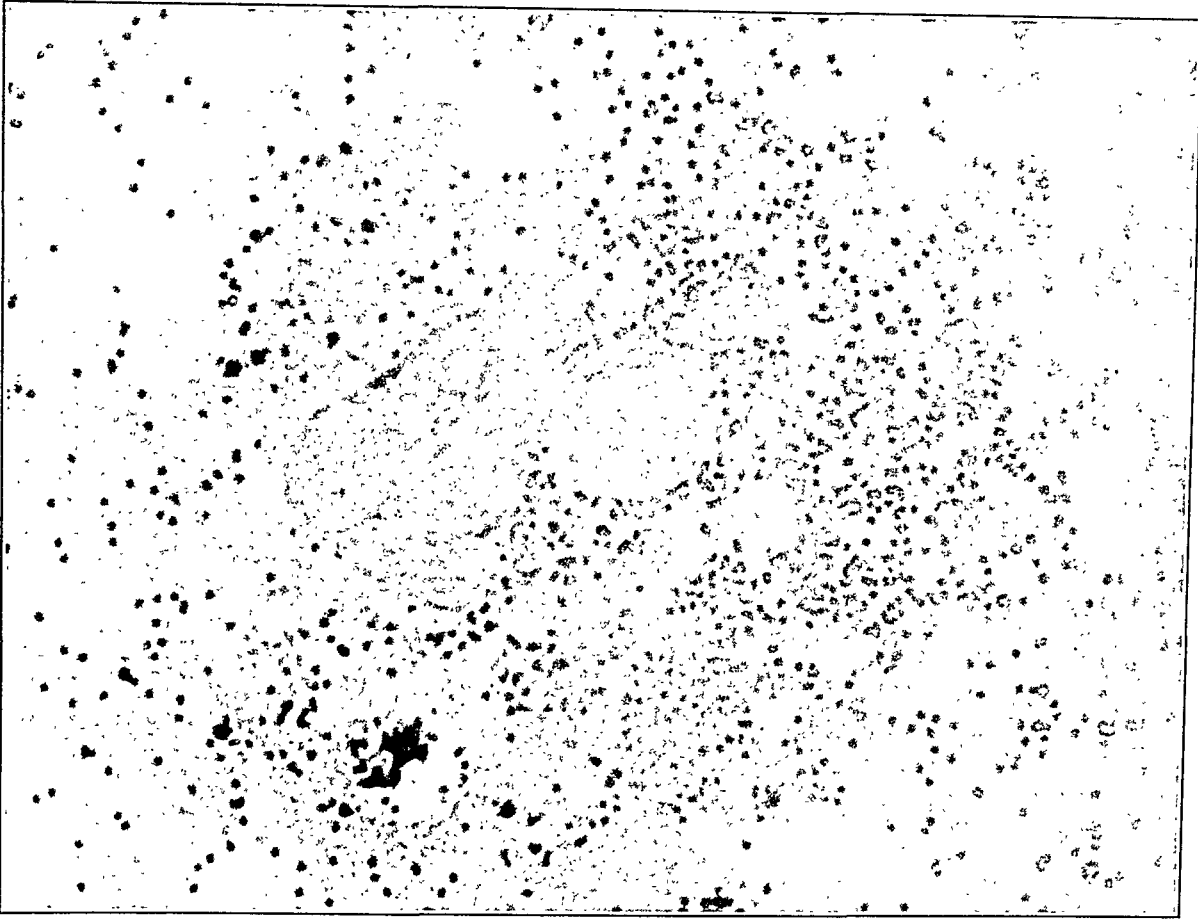
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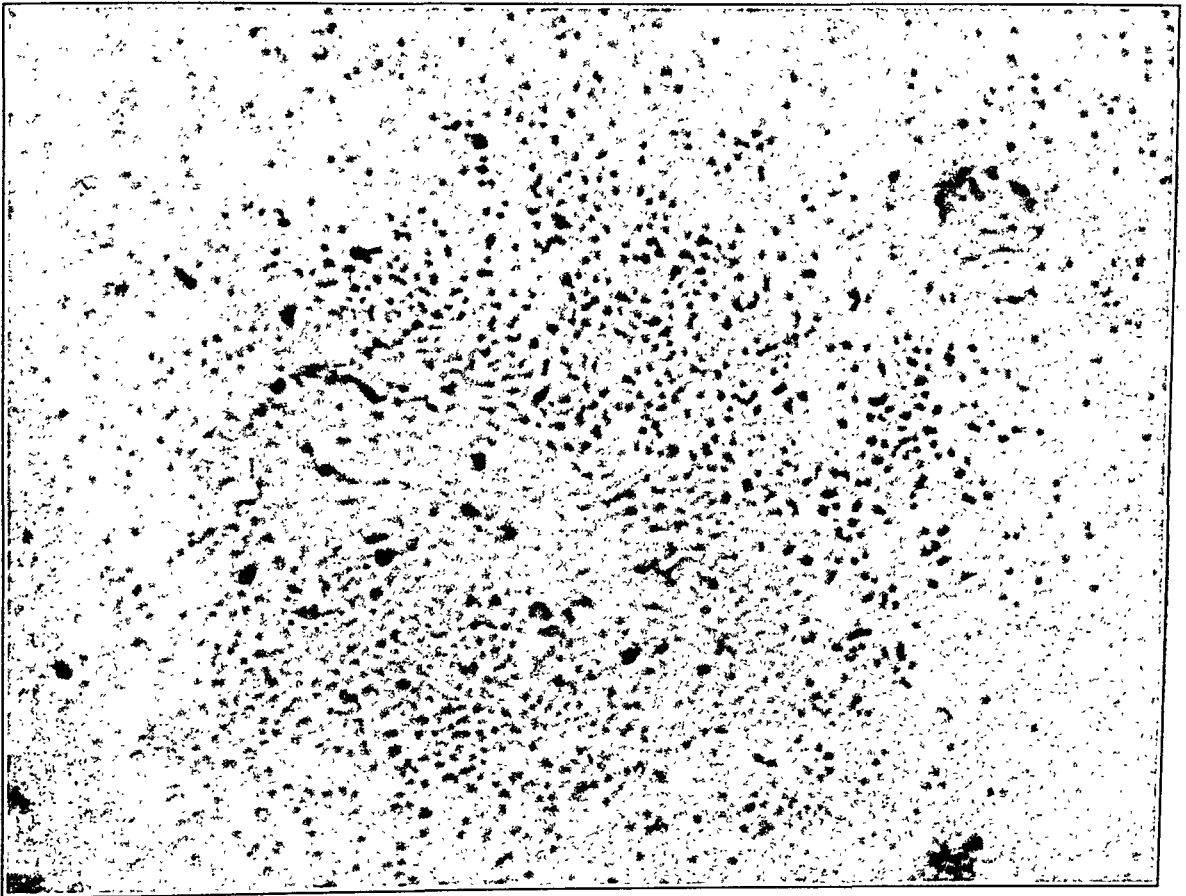
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14

and to compare these nodules with those occurring in acute rheumatic fever.

CLINICAL DATA

Material and Method: The patients studied were, for the most part, those who during the past year came to the outpatient department of the Medical School at the University of Minnesota for treatment of chronic arthritis. Subcutaneous nodules were carefully sought in 200 consecutive patients with chronic arthritis. The nodules were studied in respect to frequency, location, size, shape, consistence, development and duration. Nodules from twenty patients were removed and examined grossly. They were then cut into two or more parts under sterile conditions. A portion was fixed for microscopic study and another portion was cultured for bacterial growth. The histological structure of subcutaneous nodules found in chronic arthritis was compared with the structure of nodules of acute rheumatic origin and with experimental streptococcic nodules from rabbits.

Frequency: Dawson and Boots stated that the incidence of subcutaneous nodules in the 200 cases of "rheumatoid" arthritis examined by them was about 20 per cent. They believed that this might be higher than the general average, inasmuch as patients with subcutaneous nodules were sent to them because of their known interest in these lesions. Cecil ⁹ referred to them as being present in from 3 to 4 per cent of the cases.

In our series of 200 patients with chronic arthritis, subcutaneous nodules were found in fifty-nine (29.5 per cent). We have not attempted to determine the relative frequency of the nodules in the different classes of non-specific chronic arthritis, since we have not been able to group our arthritic patients satisfactorily into different distinct classes such as rheumatoid arthritis (atrophic, proliferative), and osteo-arthritis (hypertrophic, degenerative). Forty-eight of the fifty-nine individuals with nodules were 50 years of age or older. Some of these cases presenting definite nodules would be classified by many as osteo-arthritis (hypertrophic, degenerative). The following history illustrates such a case:

Mrs. F. P., aged 75 years, complained for the past five years of joint pains involving the toes, ankles, knee, right hip, fingers, wrists and cervical spines. The onset of illness was gradual, with moderate pain and stiffness in multiple joints. A definite nodule 1 cm. in diameter was found overlying the ulna 2 inches

SUBCUTANEOUS NODULES IN CHRONIC ARTHRITIS *

CLINICAL, PATHOLOGICAL AND BACTERIOLOGICAL STUDIES

B. J. CLAWSON, M.D., AND MACNIDER WETHERBY, M.D.

(From the Department of Pathology and the Department of Medicine, University of Minnesota, Minneapolis, Minn.)

Nodules varying in size and consistence are commonly found in the loose connective tissue beneath the skin in individuals with acute rheumatic fever. These nodules have been seen repeatedly by different observers and have come to be looked upon as a characteristic clinical and pathological finding in this condition.

Hillier¹ in 1868 was one of the first to describe these lesions. Meynet² in 1875 first pointed out that they bore a direct relation to acute rheumatic fever. Coates and Coombs³ considered subcutaneous nodules the most specific manifestation of rheumatic fever.

Not many observations have been reported concerning the frequency and structure of subcutaneous nodules seen in chronic arthritis. Hawthorne⁴ described subcutaneous nodules in six patients and considered rheumatic fever and rheumatoid arthritis different manifestations of the same process. Garrod⁵ also observed subcutaneous nodules in chronic arthritis. Wick⁶ saw a relation between the nodules found in chronic arthritis and those seen in acute rheumatic fever. Subcutaneous nodules in cases of chronic arthritis were also described by Coates and Coombs, Freund,⁷ and Dawson and Boots.⁸

If it should be shown that subcutaneous nodules are found as frequently in chronic arthritis as in acute rheumatic fever, and that the structure and etiology of the nodules in the two diseases are similar, important data would be supplied concerning the etiology of chronic arthritis. Help might also be obtained toward classifying the chronic arthritides from the etiological standpoint.

The object of this paper was to study the frequency, structure and bacteriology of subcutaneous nodules seen in chronic arthritis,

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tremities than on the lower. In fifty-nine patients nodules were found in seventy-seven locations (excluding bilateral symmetrical occurrences). Sixty-three of these locations were on the upper extremities, twelve on the lower extremities and two over the sacro-iliac joints. The distribution was as follows:

Location	Incidence
<i>Upper Extremities</i>	
Olecranon processes.....	28
Fingers (over joints)	13
Below elbows	12
Dorsum of hand.....	7
Palmar surface of hand	2
Fingers (dorsum and between joints).....	1
	—
Total	63
<i>Lower Extremities</i>	
Tibia (upper half)	4
Dorsum of foot	4
Plantar surface of foot	2
Knee (outer and lateral aspects).....	2
	—
Total	12
<i>Trunk</i>	
Sacro-iliac joints	2

Gross Appearance: On clinical examination the nodules were found to vary in diameter from 5 mm. or less to 3 cm. (Figs. 1 and 2). Those occurring over the olecranon processes tended to be small and those located below the elbow were frequently large. On superficial examination all were movable and not attached to the skin, but some were fairly firmly attached to the underlying tissues. On palpation most of the larger nodules were cystic and in some of the cysts a number of firm, disconnected masses of tissue could be felt. These masses within the cysts were occasionally attached to tendons. This was especially true on the dorsum of the hand.

The nodules were generally easily removed. They often had a definite capsule but in some instances this was poorly defined. When sectioned and examined grossly, multiple areas of necrosis surrounded by fibrous tissue were usually seen. Necrotic and mucinous material could frequently be expressed from the center. In none of the cases did we find the nodules calcified, as reported by Wick.

below the olecranon process. This nodule showed microscopically a structure similar to that found in other cases of chronic arthritis and acute rheumatic fever. The X-ray report of the right knee, right wrist, fingers, ankles, toes and cervical spines was "hypertrophic arthritis" in all except the ankles, which showed no change.

The individuals with nodules did not fall into any definite group and could not be distinguished in any way from a large number of arthritics without nodules.

Symptoms: The patients were frequently unaware of the presence of the nodules. In a few instances there was slight pain at times, especially in the nodules over the elbows when they were traumatized. Three persons having nodules on the plantar surface of the foot complained of pain in the location of the nodule and walked with a decided limp, which was definitely relieved after the nodules were removed. In one person a large nodule on the dorsum of the hand caused difficulty in extending the fingers.

Duration: In most cases it was difficult to determine the duration of disease since the patients had not known of the presence of the nodules. In a number of persons the nodules were known to have been present from a few months to as long as fifteen years. A few individuals described what appeared to have been nodules, which spontaneously disappeared after having been present from a few months to a few years.

Distribution: The nodules in this series were found chiefly on the extremities. They were not searched for as carefully on the trunk and spine in all individuals. There would seem to be no reason why nodules might not occur in such locations. Two patients had nodules in bilateral arrangement over the sacro-iliac joints. Nodules frequently occurred bilaterally in the same location, especially over and below the elbows. In one individual there were nodules similar in size over the dorsum of each hand. These nodules were attached to the lower surfaces of the tendons running to the little fingers. In another person nodules of the same size were found on the dorso-lateral surfaces of both feet, just behind the little toes. Twenty-four of the fifty-nine patients had a more or less symmetrical bilateral distribution of the nodules. This was especially true over the elbows.

Nodules were frequently multiple in the same location, especially over the olecranon processes, about the finger joints and over the tibias. They were found much more frequently on the upper ex-

in structure in different nodules and considered this variation to be due to different stages of development. He considered the primary reaction in the nodule an exudate of polymorphonuclear leucocytes, which was followed by a wandering in of round cells and by a proliferation of the surrounding connective tissue.

Swift described the nodules as being made of a conglomerate number of smaller nodules. The cellular structure was similar to the structure of nodules found in the heart and other parts of the body. Clawson¹⁷ studied nodules of acute rheumatic fever histologically in serial sections. The nodules under low magnification give the impression of multiple confluent granulomas. They are composed of multiple inflammatory foci of similar structure. The inflammatory reaction is chiefly proliferative and polyblastic in type, *i. e.*, fibroblasts and polyblasts are the most conspicuous cellular elements. Many of the polyblasts are multinucleated. Some polymorphonuclear leucocytes are found and there are irregular areas of necrosis.

Experimental Streptococcic Subcutaneous Nodules: Areas of polyblastic inflammation were observed by Small¹⁸ in a papule developing at the site of an intradermal injection of a streptococcic vaccine.

In previous experiments, in a relatively high percentage of rabbits which had been injected intradermally and subcutaneously with strains of streptococci, Clawson¹⁹ produced small, nodular, polyblastic lesions that were similar in the character of the cellular reaction to the subcutaneous nodules found in rheumatic inflammation in man.

Workers who have studied the subcutaneous nodules agree that the nodules consist of proliferating connective tissue cells and a cellular exudate of lymphocytes, plasma cells and polymorphonuclear leucocytes in varying numbers. In the center of most of the nodules there is some necrosis.

Subcutaneous Nodules in Chronic Arthritis: There have not been many microscopic examinations of subcutaneous nodules of chronic arthritic origin. Wick studied the histological structure of subcutaneous nodules from chronic arthritis and acute rheumatic fever, and found a marked similarity in the two conditions. Coates and Coombs compared the histological structure of nodules from acute rheumatic fever, from chronic rheumatoid arthritis, from Still's disease, and from a case of subacute bacterial endocarditis. They decided that the structure of the nodules in these conditions was

The nodules were grossly not unlike those sometimes occurring with syphilis and referred to as juxta-articular nodules. Subcutaneous nodules in syphilitic patients coming to the outpatient department have been found to be rare.

MICROSCOPIC STRUCTURE OF NODULES

A comparative study was made of the structure of subcutaneous nodules seen in acute rheumatic fever, of nodules produced experimentally by injecting streptococci in small doses subcutaneously into rabbits, and of nodules found in individuals with chronic arthritis.

Subcutaneous Nodules in Acute Rheumatic Fever: Hirschsprung¹⁰ in 1881 gave the first microscopic description of subcutaneous nodules found in patients having acute rheumatic fever. The nodules which he studied consisted of different modifications of connective tissue cells which varied in size and shape. The cells were irregular and spindle-shaped, and contained one or more vesicular nuclei larger than those found in ordinary granulation tissue. There was a homogeneous ground substance between the cells. The number of blood vessels was increased. He considered the nodule a localized area of inflammation which had a tendency to undergo necrosis.

Barlow and Warner,¹¹ Swift,¹² and Clawson, Bell and Hartzell¹³ observed that the cellular reaction in a rheumatic subcutaneous nodule was similar to that found in the heart valves in acute rheumatic endocarditis. Cavañy¹⁴ called attention to the presence of proliferative endarteritis in the nodules from acute rheumatic fever. This endarteritis sometimes became extensive enough to close the lumen of the vessels entirely.

The subcutaneous nodules were found by Fitcher¹⁵ to consist of fibrous tissue in various stages of development, and the cellular element to be made up of small round cells, fibroblasts, polymorphonuclear leucocytes and giant cells. Some of the giant cells contained as many as twenty-six nuclei.

Frank¹⁶ observed peripheral and central zones in the nodules. The central area was a homogeneous mass which stained red with eosin. He decided that this homogeneous material was fibrin. The peripheral zone consisted partly of spindle cells and partly of epithelioid cells. There were numerous leucocytes. He noted variation

pockets or abscesses. The nodules in these cases of chronic arthritis simulate abscesses more closely than the acute rheumatic nodules which we have studied. These necrotic, abscessed areas, since they contain streptococci, would probably tend to bring about a state of hypersensitiveness to streptococci in the patient. The small blood vessels in practically all cases show a marked perivascular increase in polyblasts, polymorphonuclear leucocytes and small lymphocytes. In some vessels the walls are thickened and the endothelium has swollen to the extent of almost obstructing the lumen. Depending apparently upon the age of the nodules, there are in most instances varying degrees of fibrosis around and about the smaller, nodular, granulomatous or necrotic areas.

The structure of the nodules from chronic arthritis does not differ in any respect, except degree, from that described in the nodules in the subcutaneous tissues, joints, tendons, galea aponeurotica, diaphragm, tongue, tonsils, arteries, heart valves, and auricles and ventricles of the heart in acute rheumatic fever. It is the structure so commonly found in acute rheumatic fever, or in nodules produced experimentally in animals by injecting streptococci. The cellular reaction in the three conditions seems probably to be due to the same cause. This type of cellular reaction is found in other streptococcic infections. The necrotic and cellular reactions and the vascular changes can be produced experimentally in rabbits and monkeys by injecting streptococci of low virulence in small numbers. The fact that subcutaneous nodules structurally similar to subcutaneous nodules in acute rheumatic fever are found in so high a percentage of cases of chronic arthritis strongly suggests a common etiology, at least in most cases.

BACTERIOLOGY OF SUBCUTANEOUS NODULES

Diplococci were demonstrated in smears from acute rheumatic subcutaneous nodules by Poynton and Paine.²⁰ Costa²¹ was able to culture streptococci from rheumatic nodules. Irish²² obtained material from rheumatic subcutaneous nodules by puncturing them with a needle. Of the six cases examined by this method he obtained streptococci in three. Swift,²³ in describing reactions produced in rabbits by injecting streptococci, referred to strains isolated from subcutaneous nodules from acute rheumatic fever. Leichtentritt²⁴ found streptococci in one of three nodules cultured.

closely related, if not identical. Freund also gave a description of the microscopic structure of nodules in cases of chronic arthritis and discussed the relation between rheumatic fever and rheumatoid arthritis. Dawson and Boots observed in their fourteen cases of rheumatoid arthritis areas of central necrosis, which they thought were due to a gelatinous swelling and disintegration of collagenous bundles. They found this necrosis to be more extensive than that found in the nodules of acute rheumatic fever. They also found fibrin and an inflammatory cell infiltration in the areas of central necrosis in some instances. Large mononuclear cells were situated around the necrotic centers, generally in a radial arrangement. They considered these cells and their arrangement to be responsible for the characteristic appearance of the lesions. The blood vessels showed subendothelial deposits of fibrin, hyperplasia of the subendothelial cells with narrowing of the lumen, and perivascular cell infiltration by large and small round cells.

We have examined microscopically subcutaneous nodules removed from twenty cases of chronic arthritis. The structure in general is similar to that described by Dawson and Boots and others.

The nodules removed are found in most instances to be made up of multiple inflammatory areas (Fig. 3). The centers of these areas commonly show varying stages of necrosis (Fig. 4). In the smaller nodules only necrotic nuclear changes such as pyknosis and karyorrhexis are most commonly seen. In the larger nodular, necrotic areas the cellular structure has disappeared. Two types of structure are seen in the necrotic centers, a hyaline, eosin-staining material and a fibrillar substance that stains with hematoxylin. Scattered in this necrotic material are varying numbers of polymorphonuclear leucocytes. Surrounding the necrotic centers there are many mononuclear and multinucleated cells (polyblasts). These vary in size and shape. Many of them resemble the epithelioid cells in a tuberculous lesion. These polyblasts generally, but not always, have a marked tendency to be arranged in a radial or palisade fashion (Figs. 5 and 6). In this respect the arrangement is similar to that so commonly found in the heart valve in acute rheumatic endocarditis. A hyalinized material, similar to that seen in the valve in acute rheumatic endocarditis, is often seen (Fig. 3). Scattered among the larger and irregularly shaped polyblasts are small polyblasts. Polymorphonuclear leucocytes are scattered among the polyblasts, in many cases in small

Structurally these nodules seemed to be similar to those found in acute rheumatic fever and to those produced in animals by injecting streptococci. They tended to be larger in chronic arthritis. The microscopic structure was chiefly polyblastic in character, but small areas of necrosis and abscess formation were commonly seen.

When these nodules were macerated under sterile conditions and cultured in beef infusion dextrose broth, diplococci morphologically and culturally similar to those commonly found in the blood of patients having acute rheumatic fever or chronic arthritis were isolated in 70.6 per cent of the cases.

The frequency of the subcutaneous nodules in acute rheumatic fever and chronic arthritis, the similarity of the gross and microscopic structure of the nodules in these two conditions and in experimental streptococcic nodules, and the frequency with which streptococci can be cultured from the blood in acute rheumatic fever and from the blood and nodules in chronic arthritis, strongly suggest that acute rheumatic fever and chronic arthritis for the most part have a common streptococcic etiology and that the two diseases are in all probability different manifestations of the same process.

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Previous to this report we attempted to culture bacteria from three subcutaneous nodules of rheumatic origin, but failed to obtain growth. Billings, Coleman, and Hibbs,²⁵ found *Streptococcus viridans* in a "fibroid nodule" from a patient with chronic arthritis. The location of the nodule was not given in their report. Aside from this report, no positive cultures from subcutaneous nodules in chronic arthritis have been reported. Wick was not able to obtain organisms from the nodule which he cultured. Dawson and Boots cultured nodules from fourteen patients with chronic arthritis, but had negative findings in all cultures.

We removed the nodules under sterile conditions from twenty of our patients. Seventeen of these were cultured. The nodules were brought to the laboratory immediately in sterile test tubes. They were then placed in sterile Petri dishes and cut into parts with sterile scissors. The part to be cultured was further macerated by cutting it several times with the scissors. The macerated material was then placed in a test tube containing about 10 cc. of beef infusion broth, which was similar to that used for culturing the blood from patients with acute rheumatic fever and chronic arthritis. This broth had a reaction of pH 7.6 and contained 0.2 per cent of dextrose. The material in the broth was incubated at 37° C and examined by smear preparation about three times a week. Growth, when present, occurred as a rule in less time than in blood cultures; however, cultures were not discarded until they had been incubated for at least one month. Of the seventeen nodules from different patients cultured by this method streptococci were recovered in twelve (70.6 per cent). *Staphylococcus albus* was obtained from one. Some of the strains grew well and some grew poorly. Morphologically the strains as a rule were diplococci, though two strains grew out into chains of diplococci. All were Gram-positive and produced green discoloration faintly when grown on a sheep blood agar plate for twenty-four hours at 37° C.

SUMMARY AND DISCUSSION

Two hundred patients having chronic arthritis were studied to determine the frequency of subcutaneous nodules in this disease. Nodules varying in diameter from 5 mm. to 3 cm., and in number from one to several per patient, were found in 29.5 per cent of the cases.

DESCRIPTION OF PLATES

PLATE 46

FIG. 1. Subcutaneous nodule on arm below elbow.

FIG. 2. Large subcutaneous nodule on right side of foot.

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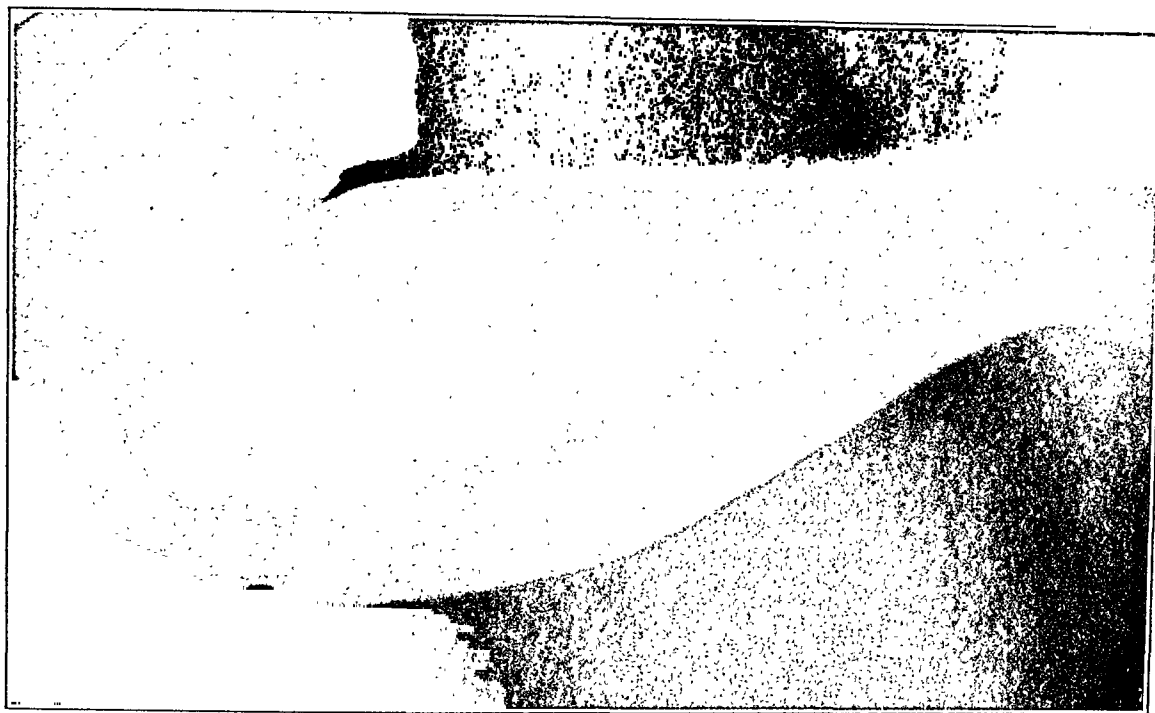
PLATE 47

FIG. 3. A small subcutaneous nodule and part of a larger one lying near each other. The smaller nodule shows a hyalinized material and cellular content similar to that in a valve in acute rheumatic endocarditis.

FIG. 4. A nodule showing a necrotic center with radial arrangement of polyblasts around the necrotic area.

FIG. 5. Cellular content of a nodule against the necrotic center on the right.

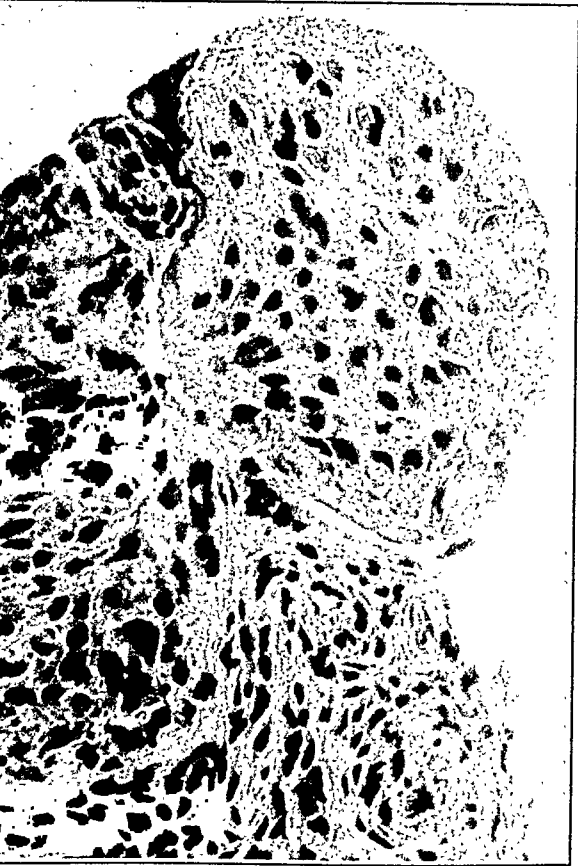
FIG. 6. Palisade arrangement of cells against the necrotic center in the nodule.



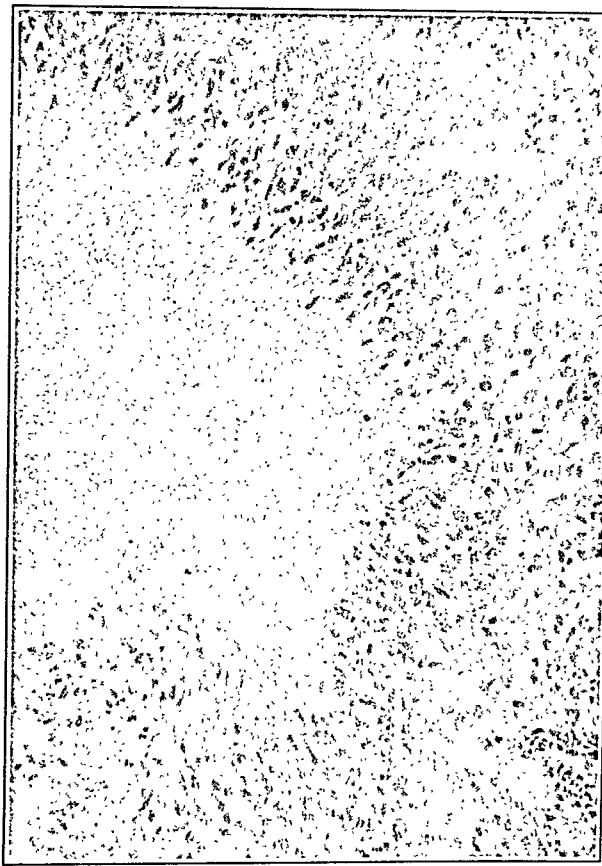
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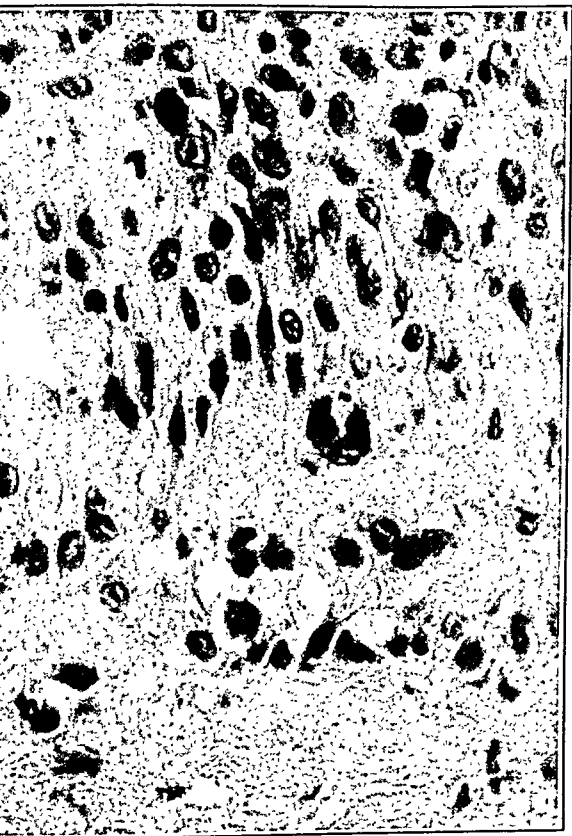
2



3



4



5



6

3. Gallocyanin (oxyproduct of the oxazine group).
4. Gallamin blue (derivative of No. 3).

As lack-forming salts we used:

$\text{Al}_2(\text{SO}_4)_3$
 K_2SO_4 , Cr_2SO_4 , 24 H_2O (chromalum).
 Al Cl_3
 K_2SO_4 , $\text{Al}_2(\text{SO}_4)_3$, 24 H_2O (potassium alum).
 $\text{Na}_2 \text{B}_4 \text{O}_7$ (borax).

The salt is dissolved in distilled water, whereupon the dye is added and mixed thoroughly with the solution. The mixture is then warmed gradually and gently and finally allowed to boil for 15 to 25 minutes, the bottle being shaken frequently. After gradual cooling and filtration the solution is ready for use. In the case of Naphthazarin and Alizarincyanin R the solution is allowed to stand for about 8 days and then refiltered.

The following dye solutions were employed:

1. Naphthazarin-aluminum sulphate 5%, pH = 3.23

$\text{Al}_2(\text{SO}_4)_3$	10.	gm.
Distilled water	200.	cc.
Naphthazarin	0.35	gm.

Color of solution: intense, deep reddish violet.
 Staining time: 20 to 40 hours.
2. Alizarincyanin R-aluminum sulphate 5%, pH = 3.43

$\text{Al}_2(\text{SO}_4)_3$	10.	gm.
Distilled water	200.	cc.
Alizarincyanin R.....	0.3	gm.

Color of solution: deep violet with reddish tint.
 Staining time: 20 to 30 hours.
3. Alizarincyanin R-aluminum chloride 5%, pH = 3.09

Al Cl_3	10.	gm.
Distilled water	200.	cc.
Alizarincyanin R	0.3	gm.

Color of solution: deep violet with a somewhat stronger red than Solution 2.
 Staining time: 20 to 30 hours.
4. Alizarincyanin R-chromalum 5%, pH = 1.87

Al Cl_3	10.	gm.
Distilled water	200.	cc.
Alizarincyanin R	0.3	gm.

Color of solution: dark blue with a gray tint.
 Staining time: 24 to 60 hours.

A METHOD FOR PROGRESSIVE SELECTIVE STAINING OF NISSL AND NUCLEAR SUBSTANCE IN NERVE CELLS *

LARUS EINARSON, M.D.

FELLOW OF THE ROCKEFELLER FOUNDATION

(From the Anatomical Institute, Munich, Pathological Laboratory of the Bispebjerg Hospital, Copenhagen, and the Marine Biological Laboratory, Woods Hole, Mass.)

The usual staining methods for the demonstration of Nissl substance suffer from a variety of technical defects and difficulties. I have succeeded in devising a progressive selective method for staining this substance, which I believe to be of both practical utility and of some theoretical importance.

TECHNIQUE

The Nissl substance is a constant histological element in nerve cells, which has a marked affinity for basic dyes. As yet, however, every method for demonstrating it has depended upon the "regressive principle of staining," *i. e.*, overstaining and then differentiating in alcohol. The basic anilin dyes have been the most useful, and yet generally speaking they do not stick firmly enough to the tissue elements and are too readily removed by alcohol. In this respect there is only a difference of degree between these various dyes. It is clear that the differentiation profoundly influences the results, and the hurry with which the preparation must be passed through the alcohols and xylols is a great disadvantage. Following an extensive study of the literature I arrived logically at the following method, which eliminates nearly all of the above difficulties.

Becher¹ introduced the use of certain dyes from the groups of anthraquinones, naphthoquinones and oxazines. These dyes are combined with a metallic element forming a soluble "lack," and this now basic dye becomes bound by the fixed acid nuclei (see also Romeis²).

The dyes † we have used are:

1. Naphthazarin (1, 2 — dioxynaphthoquinone).
2. Alizarincyanin R (1, 2, 4, 5, 8 — pentaoxyanthraquinone).

* Received for publication January 8, 1932.

† Nos. 1, 3 and 4 were obtained from Dr. Hollborn (Grübler-Hollborn, Leipzig), and No. 2 from Dr. G. Grübler & Co., Leipzig.

In No. 1 the tissue was fixed up to maximum consistency in 5 to 6 days with frequent changing of fluid, in No. 2 about 24 to 48 hours, in No. 3 — 24 hours, in No. 4 — 8 to 12 hours, in No. 5 about 20 hours (medulla oblongata of dog), in No. 6 about 3 to 4 days. Then followed the usual procedures for dehydration, and so on. All the material was embedded in paraffin through methylbenzoatcelloidin and benzol after the method of Péférfi. The slides (sections 5 to 10 microns thick) are placed directly from distilled water into the dye solution in question. By varying the duration of staining the intensity of color can be graded somewhat, but overstaining practically never occurs. In general, old solutions (several months) require a longer time for staining than fresh ones.

After staining, the specimen is carefully washed in distilled water, dehydrated in alcohols of increasing concentrations in xylol, and mounted in balsam, damar-resin or cedar oil. When the stained slides are run through the alcohols there is no danger of their losing color. The staining is absolutely alcohol-stable and differentiation does not occur.

DESCRIPTION OF RESULTS

Figs. 1, 2, 3, 5 and 9 illustrate the results obtained with Solutions 1 and 2. Stained with Naphthazarin (Solution 1), the Nissl granules appear blue-violet, and more deeply colored than with Alizarincyanin (Solution 2) where they are reddish and not so distinct. The nerve cell in Fig. 9 appears faint and somewhat washed out. In Solution 1 the co-staining of the interstitial protoplasm of the single nerve cells, as well as of fibrillar structures, is violet, in Solution 2 more reddish. Otherwise the intensity of the co-staining seems to be approximately the same in both instances. Examining Fig. 5, where the staining time is so much longer, the co-staining is correspondingly greater than in Figs. 1 and 2. Also, the interstitial protoplasm and the karyoplasm in general (Kernsaft) are more strongly colored, yet the nucleolus is easily distinguishable. The picture appears more diffuse. On the whole the Naphthazarin method yields a very good staining for general orientation and for the arrangement of the Nissl substance.

With Alizarincyanin (Solution 3) we get a much more intense color (Fig. 10). The Nissl substance is a deep red-violet color, the karyoplasm is relatively light, the nuclear membrane can be seen.

5. Gallamin blue-potassium alum 5%, pH = 2.43

Potassium alum	10.	gm.
Distilled water	200.	cc.
Gallamin blue	0.4	gm.

Color of solution: deep, dark blue.

Staining time: 24 to 48 hours.

6. Gallocyanin-chromalum 5%, pH = 1.84

Chromalum	10.	gm.
Distilled water	200.	cc.
Gallocyanin.....	0.3	gm.

Color of solution: deep, intense blue, with a faint violet tint.

Staining time: 24 to 48 hours.

7. Gallocyanin-chromalum 2%, pH = 1.89 (*i. e.* chromalum 4 gm.)

Color of solution: approximately the same as in Solution 6.

Staining time: 24 to 48 hours.

8. Gallocyanin-borax 2.5%, pH about 9 (*i. e.* borax 5 gm.)

Color of solution: deep, intense blue; after standing for about 12 days, a marked reddish brown tint appears.

Staining time: 24 to 48 hours.

The pH of the solutions was determined with a quinhydrone electrode. The absolute values are less significant than the constancy for each solution and the differences between them. The figure for Solution 8 is naturally inexact, the solution being too alkaline for this method.

The animals used were killed by cutting both carotids under light ether anesthesia and allowing the animal to bleed to death. The desired part of the central nervous system was quickly exposed and small pieces (about 5 to 8 or 10 mm. long) cut out and immediately put into the fixing fluid.

For fixation were tried:

1. 96% alcohol, after the method of Nissl.
2. Alcohol-formalin (90 vol. 70% alcohol plus 10 vol. of the usual 40% solution of formaldehyde after being neutralized).
3. Sublimate-alcohol.
4. Formol-alcohol-sublimate (F. A. S.) (1 vol. 40% formalin, 4 vol. 93% alcohol, 5 vol. saturated aqueous solution of sublimate).
5. Zenker's fluid with acetic acid.
6. Neutral formalin (1 part formalin, 4 parts distilled water).
7. Susa (Heidenhain).

The fluids Nos. 1 to 4 gave excellent results and with a little longer duration of staining No. 5 also gave splendid results. No. 6 gave the same excellent pictures, but No. 7 gave a somewhat weaker staining.

Any of the usual methods of after-staining may be applied to specimens stained by the methods we have described. For example, we may use Alizarin-red S for 3 to 8 minutes, followed by washing in distilled water and so on; or erythrosin (0.05 per cent) or eosin (0.05 per cent) for 5, 15 or 60 seconds, followed by washing and differentiation in graded alcohols and so on. We hope that this staining method, being so easy and simple, may prove of practical value, particularly to neuropathology.

Somewhat similar staining experiments have been made by Kihn³ but with totally negative results. His work has even been quoted as showing the great limitations of this staining principle as applied to the nervous system. Just what factors underlie his negative results, I am so far unable to tell. Apparently he has used solutions containing only 2 per cent chromalum. Therefore I tried Solution 7. Macroscopically the slides show a stronger staining as compared to specimens from the same block stained in Solution 6; apparently more dye has been absorbed. Microscopically this is due to a greater general co-staining, especially of fibrillar material. In the individual nerve cell there is also a somewhat stronger staining of interstitial protoplasm and the karyoplasm, but there is no alteration in the staining of the Nissl substance itself. This possibly indicates (the pH being the same in both cases) that the dye has become less completely bound to the chromalum to form the compound dye-lack, and consequently is to a certain extent acting as an acid dye.

The alkaline solution (8) was tried on two differently fixed preparations from the spinal cord of a rabbit. The first specimen fixed in formalin-alcohol looks macroscopically as if there were no staining at all. Microscopically the only things that are brought out are the Nissl bodies and nucleolus of the nerve cells and the glia nuclei. These appear as weak, gray shadows, faint, but distinctly visible. Sometimes one also sees the nuclear membrane. The other preparation, fixed in trichloroacetic acid, gives an entirely different picture. Macroscopically the slides appear relatively deeply stained — a uniform gray-blue. Microscopically there is considerable general staining of fibrillar material, and so on. The protoplasm of the nerve cells is gray, and the nucleus is considerably darker with a clearly distinguishable nucleolus. There is not a sign of Nissl bodies. This illustrates very well the significance of the fixative and the well known importance of its acidity or alkalinity.

The interstitial protoplasm of the nerve cell is strongly stained in the same reddish color but, owing to the intense darkness of the tigroid, the method yields a certain contrast which makes it fit for use. The glia nuclei are markedly colored, but do not stand out particularly because of the strong co-staining of fibrillar structures. The general impression of this staining is the impurity of the picture as a whole.

With Solution 4, where the same dye is in combination with chromalum, one gets a picture of entirely different character, both in color and intensity. After 53 hours of staining (Fig. 11), glia nuclei, the Nissl substance and nucleoli of the nerve cells are but faintly light blue or water-blue. There is also a weak co-staining of the interstitial protoplasm of the nerve cells and of fibrillar structures. Practically the staining is not of much value.

Gallamin blue (Solution 5) yields a fine, pure, bright blue staining of the tigroid (Fig. 12). The staining comes next to the Gallocyanin-chromalum in color and purity, but the blue co-staining is considerably more extensive and of approximately the same intensity as with Naphthazarin. Yet the staining is of practical value.

By using Gallocyanin (Solution 6) one gets Nissl pictures of a quality unapproached by any other method. I have tried and used all of them, Nissl's original method included. Even the finest, most carefully made thionin or toluidin blue specimens do not approach the Gallocyanin specimens, the force of which lies in the progressivity and selectivity of the staining. The Nissl substance (Figs. 4, 6, 7 and 8) appears deeply stained in the purest and finest blue color, giving an excellent sharp contrast against the practically unstained interstitial protoplasm. The nucleolus stands out sharply from the pale nucleus. Glia nuclei are also beautifully stained, giving a good contrast against the pale background. In other words, the general co-staining of fibrillar structures, and so on, is practically minimal. A glance at the figures shows the superiority of this method to those previously described. The beautiful appearance of the nuclear cap (Kern Kappe) in Fig. 4 is noteworthy. On examining the pathological case of Fig. 8 one notices how sharply and beautifully every detail is brought out — the central degeneration with tigrolysis, accumulation of chromophile material at the periphery of the cell, the vacuolization, and so on.

DISCUSSION

To indicate the theoretical significance of this method in regard to the origin and composition of the Nissl substance we need consider only a few necessary data from the literature on the subject. It will appear that the color reaction of the Gallocyanin is not entirely devoid of theoretical importance.

Mackenzie⁵ found that the Nissl substance contained iron, and finally stated definitely that it was an iron-holding nuclear chromatin. Held⁶ found that it gave a positive reaction for phosphoric acid, and classed it as a nucleoproteid. Scott,⁷ applying the methods of Macallum, found the Nissl substance to give a positive reaction for both iron and phosphorus. On the basis of his embryological investigations he further states that the iron-containing material (Nissl substance) is gradually formed by transfer of nuclear substances through the nuclear membrane into the cytoplasm during the course of development. The nuclei of embryonic nerve cells he found to be much richer in basophil chromatin material than after the Nissl substance had become differentiated. Recently Nicholson,⁸ using Macallum's hematoxylin test for iron, has been able to show that the distribution and appearance of iron-containing material in the cytoplasm is practically identical with the distribution and appearance of the Nissl substance. Previously von Lenhossek⁹ had said that the tigroid is already present in the young neuroblasts in the form of smaller and more diffusely arranged spheroidal masses. Collin¹⁰⁻¹² confirms Scott's observations and states that he has been able to observe in his preparations all the phases involved in the process of transfer of chromatin material from the nucleus into the protoplasm. He further finds that the chromatophil substance first appears in the immediate neighborhood of the nucleus of the nerve cells of chick embryos of about six days' incubation. Marcora¹³ states that the differentiation of chromatophil granules is first observable on the tenth day of incubation, always appearing first in the peripheral part of the cellular protoplasm. Beyond this he does not confirm Collin's observations, and he believes that the question of the nuclear origin of the chromatophil substance cannot yet be solved. According to van Biervliet's investigations on human material (cited from Marcora) the first Nissl granules become differentiated in the third month, appearing at the cell periphery; but the differentiation is by

An alteration in the pH of the dye solution was also tried. In order to do this N/10 HCl or NaOH were added to the Galloccyanin-chromalum solution, the determination of pH made quickly, and immediately afterward the specimen in question put into the solution for staining.

Solution 6-(original)	gives pH = 1.84
Solution 6-55 cc. plus N/10 HCl 30 cc.	gives pH = 1.58
Solution 6-40 cc. plus N/10 NaOH 15 cc.	gives pH = 3.57
Solution 6-42 cc. plus N/10 NaOH 35 cc.	gives pH = 4.57

In doing this the dye solution was diluted, but this does not seem to be of any great importance in this connection. The results, though revealing nothing that could explain Kihn's negative results, were rather interesting. With fixative No. 2 we find macroscopically a characteristic gradation of color intensity, the most acid solution giving by far the lightest, the least acid solution giving the darkest stain. The amount of stain absorbed in general by the tissue is, as one would expect (see Pischinger ⁴ 1926), a function of the pH of the dye solution, all preparations having been fixed in the same way. Microscopically one sees that this is due to the general co-staining. In the specimen stained in the solution of pH = 1.58 there is practically no co-staining at all. With the usual solution of pH = 1.84, the co-staining is just perceptible. pH = 3.57 gives a considerably stronger co-staining, and pH = 4.57 still greater. Between the first and the last solutions there is an enormous difference, but the most interesting thing is the comparatively slight change in the staining of the Nissl substance itself, in striking contrast with the general co-staining. That is to say, the specific tigroid and chromatin staining is practically unaffected by this alteration in pH of the dye solution. The results with fixative No. 1 are almost identical.

In general, the compound Galloccyanin-chromalum, being a basic dye, is more readily absorbed in a less acid solution than in a more acid one, this effect manifesting itself microscopically through alterations in the general co-staining of the tissue which is clearly a direct function of the pH of the solution; whereas the specific Nissl and chromatin picture is relatively unaffected within the same range of variation of pH.

nerve cells and have been long known there as Nissl bodies. As a support of this view they refer to some cases of identical behavior of these substances in epithelial cells, plasma cells, liver and nerve cells. As a supplement to these positive points they give the negative microchemical proof that the Nissl bodies do not contain nuclein. The basis of this proof is their assumption that purified methyl green is an absolutely reliable reagent for distinguishing between nuclein and other acid protein substances in the cell; but the lack of specificity of this reaction has been pointed out (see Cowdry¹⁵). They also find that Nissl substance dissolves in warm water (65°) in twelve hours and believe it to be albumose (cytose).

Heidenhain³⁰ has advanced a very interesting and stimulating hypothesis on the biological function of the Nissl substance, based on the assumption that it is derived from the nucleus and contains chromatin substance, and he suggests the name "Cytochromatin" for the Nissl granules.

Stimulated by Heidenhain's hypothesis and assuming that Mackenzie, Held, Scott and Nicholson were essentially correct in their views as to the chemistry of the Nissl granules, it seemed to me quite plausible that the Nissl substance should manifest an affinity for, and be stainable progressively with, some of the purest and finest nuclear dye-lacks from the group of anthraquinones, naphthoquinones and oxazines. Our results, which demonstrate just such an affinity, serve to support the assumption that the Nissl substance is related to chromatin, and to oppose the views of Unna and Gans. In this connection I wish to emphasize the distinct gradation in the character, intensity and purity of the staining according to what dye combination is being used. This gradation shows a distinct parallelism between the quality of the staining of true nuclear chromatin material and that of the Nissl substance, *i. e.*, *the purer the affinity for nuclear chromatin the stronger appears to be the affinity for the Nissl substance.*

The problem of the nature of Nissl substance presents the following alternatives. It may be (a) a product of nuclear activity formed during the life of the developing neurone and fulfilling a function of importance in the metabolism of the neurone; or (b) a preformed material (präformierten Stoff, (Unna)) arising outside of the nucleus during ontogenesis, able to reform or regenerate during life; or (c) an artefact containing nuclear substances (de Moulin). This

no means perfect until in the seventh month of fetal life. Cowdry¹⁴⁻¹⁷ definitely states that the Nissl substance belongs to the category of chromidial substances in general, and infers that it is formed as the result of nuclear activity. All the investigators seem to agree that the Nissl substance appears relatively very late in development.

Mott¹⁸ has been able to confirm the ultramicroscopic observations of Marinesco¹⁹⁻²². After vital staining and treatment with formalin he showed further that the cytoplasm became filled with minute blue granules. He therefore draws the conclusion that the minute refractive granules make up the basophil substance which forms the Nissl substance (Schollen), while Marinesco himself considers them to be identical with the neurosomes of Held. The Nissl figures (Schollen) as such, both Mott and Marinesco consider to be artefacts. On the contrary Stöhr,²³ on the basis of investigation with ultraviolet photomicrography, claims to have observed in fresh condition the same localization and grouping of the Nissl granules in the cytoplasm as in fixed and stained preparations. This is also confirmed by Weimann.²⁴

De Moulin,²⁵ using as fresh material and as well adapted technical arrangements as possible, added methylene blue to his emulsion and found no basophily of the cytoplasm, but the nucleus as well as nucleolus became deeply stained. Gradually the nucleus lost its stain, while the cytoplasm, on the other hand, gained in color and slowly became a diffuse homogeneous blue. Granulations then appeared, and at last regular Nissl figures (Schollen). His conclusions are that postmortem changes in the degree of dispersion in cell and nucleus occur, causing a shift in the fluids; and following lesions of the nuclear membrane, the basophil colloids of the nucleus get into the protoplasm where they coagulate, and thus finally form the Nissl substance. The latter he therefore considers as an artefact composed of nuclear substances.

Recently a systematic criticism has been made of the methods for microchemical detection of phosphorus, and therefore Scott's results must be considered with some reservation (see Bethe²⁶ and Policard and Leulier²⁷). Bielschowsky,²⁸ and several others, deny any connection between nuclear substances and the Nissl granules. The most outspoken critics are Unna and Gans.²⁹ They state that the substances widely distributed in epithelial and connective tissue cells, and described by Unna as "Granoplasma" are also present in

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problem I leave open for the present, being engaged in further investigation upon this question.

CONCLUSIONS

Well aware of the danger and difficulties of deducing from mere similarity of dye affinities the identity or chemicobiological relations of two tissue elements, I nevertheless believe that the positive Gallo-cyanin reaction furnishes new evidence that the Nissl substance really contains nuclear chromatin substances. The results of the chemical, the embryological and the experimental investigations summarized above, and certain characteristics of the Gallocyanin reaction, definitely lend support to such a view.

The specific Nissl staining of the Gallocyanin-chromalum is practically unaffected within a very wide range of pH of the dye solution, and also in combination with borax in an alkaline solution it still stains the chromatin and Nissl substances feebly but definitely. This indicates that possibly some chemical affinities are involved in this special staining process. Further investigation of this point is in progress.

The cytological work leading to the method described here was started during my stay in the Anatomical Institute of Munich, continued in Doctor Vimtrup's laboratory in Copenhagen, and greatly extended and completed in the Marine Biological Laboratory of Woods Hole this summer.

I wish to express my gratitude to the director of the Anatomical Institute of Munich, Geheimrat Professor Mollier and to Prosector Doctor Vimtrup for their great hospitality and supply of material. Also I wish to thank Doctor Neel, the director of the Psychiatric Laboratory of Copenhagen, for his kindness in supplying me with vast, special neuropathological material upon which I have tested the practical pathological validity of the staining method.

Above all I want to express my deep gratitude to my friend and teacher Dr. Robert Feustel of Munich for his many valuable suggestions, stimulating ideas and never-failing interest.

And last I wish to express my thanks to Dr. Hallowell Davis for his revision of this paper and valuable advice.

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DESCRIPTION OF PLATES

PLATE 48

- FIG. 1. Cells from the spinal cord of a rabbit; formalin-alcohol, Naphthazarin- $\text{Al}_2(\text{SO}_4)_3$. Staining time: 23 hours, 25 minutes. $\times 860$.
- FIG. 2. Anterior horn cell of a rabbit; formalin-alcohol, Naphthazarin. Staining time: 23 hours, 25 minutes. $\times 860$.
- FIG. 3. Purkinje cell, from the cerebellum of a rabbit; neutral formalin, Naphthazarin. Staining time: 23 hours, 25 minutes. $\times 860$.
- FIG. 4. Purkinje cells, cerebellum of a rabbit; neutral formalin, Gallocyanin-chromalum. Staining time: 22 hours. $\times 860$.

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PLATE 49

- FIG. 5. Anterior horn cell, spinal cord of a dog; 96 per cent alcohol, Naphthazarin. Staining time: 40 hours. $\times 960$.
- FIG. 6. Anterior horn cell, spinal cord of a dog; 96 per cent alcohol, Gallo-cyanin. Staining time: 25 hours. $\times 960$.
- FIG. 7. Anterior horn cell, spinal cord of a rabbit; formalin-alcohol, Gallo-cyanin. Staining time: 24 hours. $\times 1030$.
- FIG. 8. Anterior horn cell, spinal cord of a child; poliomyelitis anterior acuta; Gallo-cyanin. Staining time: 24 hours. $\times 1030$.



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PLATE 50

FIG. 9. Anterior horn cell, rabbit; formalin-alcohol, Alizarincyanin- $\text{Al}_2(\text{SO}_4)_3$. Staining time: 25 hours, 30 minutes. $\times 860$.

FIG. 10. Cell from the medulla oblongata, rabbit; formalin-alcohol, Alizarincyanin- Al Cl_3 . Staining time: 25 hours, 30 minutes. $\times 860$.

FIG. 11. Cell from the medulla oblongata, rabbit; formalin-alcohol, Alizarincyanin-chromalum. Staining time: 53 hours. $\times 860$.

FIG. 12. Medulla oblongata, rabbit; formalin-alcohol, Gallamin blue. Staining time: 25 hours, 30 minutes. $\times 860$.



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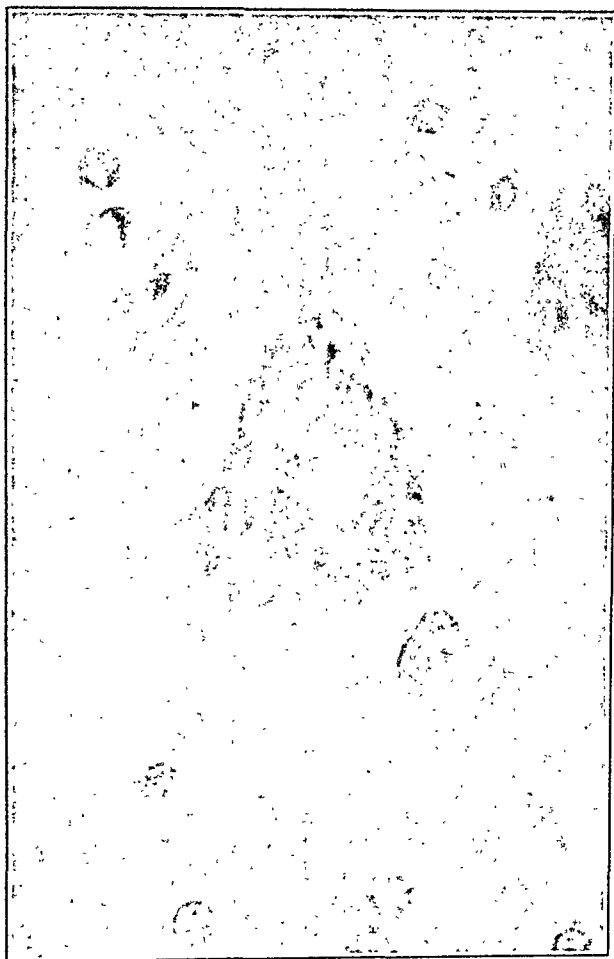
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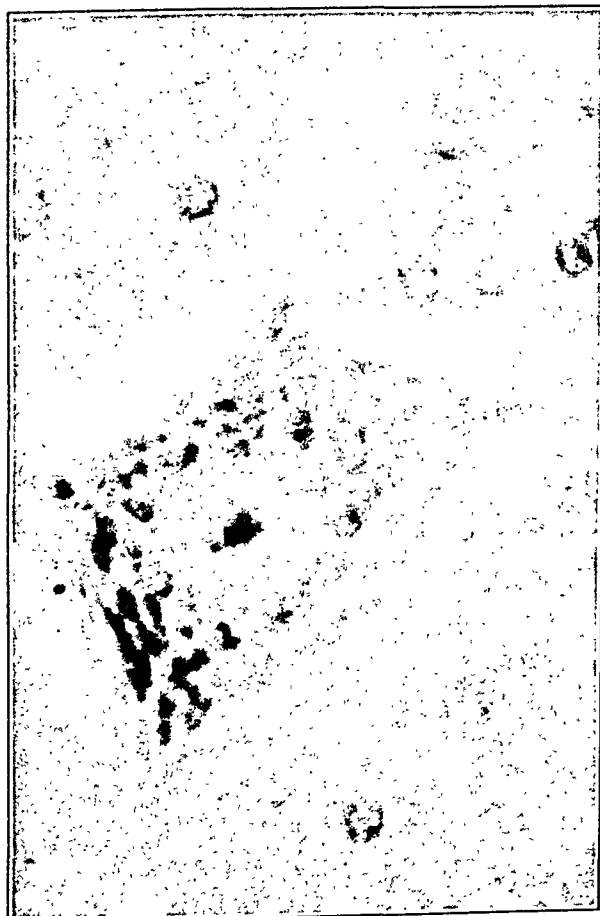
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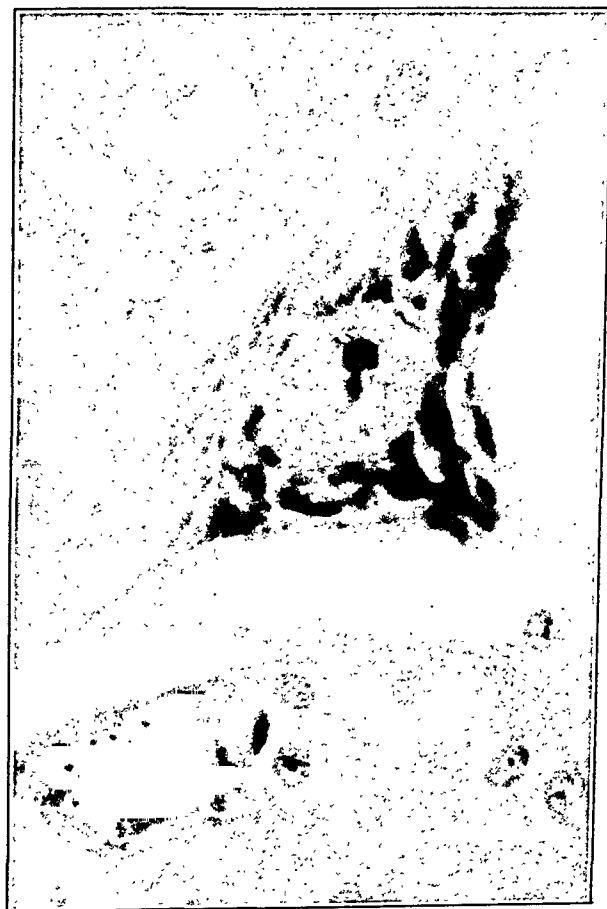
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Einarson

Method for staining Nissl and Nuclear Substance

of Merkel-Ranvier cells on the other (in the former case the cells are large and pale, in the latter they are smaller, polyhedral and possessed of protoplasmic processes); in the deeper layers of the tumor these cells become elongated into fusiform, or even filamentous structures that are closely applied to the nerve fibers and may be flattened into concentric plates that are, in turn, enclosed in a collagenous envelope or endoneurium that ramifies among them and gives them an onion-like, laminated appearance on cross-section. When the tumor cells become so attenuated as to be filamentous he calls them "nevus fibers," which is rather misleading and apt to be misunderstood, as they are really merely elongated cells rather than true fibers. Transitions between the superficial and deep types of growth may be observed in the intermediate layers of the tumors.

Such newgrowths, then, lie directly in the course of the terminal branches of the cutaneous nerves and are, therefore, closely related to neurofibromas. The pigmented cells, formerly considered so important as to give their name to the tumors, he explains on the basis of a changed metabolic activity; the fact that two or more types of cell contain melanin is no proof that they are necessarily genetically related. In support of this he cites the presence of melanin-bearing cells in other parts of the nervous system. The part played by the specialized Merkel-Ranvier cells of the epidermis is explained by the assumption that they are outposts of the tactile apparatus — potential outposts of nevi — intercalated between the true epithelial cells of the basal layer and of another cell race than these. He speaks of all the tumor cells as constituting an overgrowth of the "peripheral neuroglia," doubtlessly basing his terminology upon the theory that the Schwann cells are of epiplastic, neuroglial origin.

Having thus outlined his views as to the histogenesis of the tumor, he indicates very clearly that it is unprofitable to attempt any further classification of its subtypes, as was formerly the fashion. If his conception be understood, it will readily be seen that tumors developing in the deep layers of the skin will take on the complicated neurofibromatoid type of growth, while those arising just beneath the epidermis will tend to be cellular and alveolar, the type cell varying as it inclines now toward those of the Merkel-Ranvier group, now toward those of the Meissner corpuscles. Tumors developing in both the pars papillaris and the derma will show a mix-

CONCERNING THE HISTOLOGY OF MELANOMA *

NATHAN CHANDLER FOOT, M.D.

(From the Department of Pathology, Cincinnati General Hospital and College of Medicine of the University of Cincinnati, Cincinnati, Ohio)

INTRODUCTION

Certain features of melanotic tumors, noted in frozen sections impregnated with silver, were discussed at some length in a recent paper (Foot ¹). The point most stressed was the presence of a very complex reticulum in the tumors examined, the fibers of which were described and reproduced photomicrographically. The origin of these fibers, their probable nature and their ultimate distribution, were points reserved for later and fuller discussion, as it was not then possible to impregnate paraffin sections satisfactorily, and these are indispensable. Now that a very satisfactory method has been devised, it is proposed to carry the discussion farther and to supplement that which has already been said with a description of new data that have resulted from the study of thinner, more perfect sections. Before entering into the details of the matter, it is advisable briefly to review the subject in respect to that which has already been advanced by another investigator, whose work inspired these studies.

Two articles published by Masson ² in 1926 have covered this theme so thoroughly that, in checking over his work by means of other methods than his, one may at best hope for little more than a confirmation of his findings. The more one reads his papers, the more one is impressed with their completeness and clear-cut logic. His conception of the histology of the pigmented nevi and of their malignant congeners, the melanoblastomas, is none too readily grasped at first reading and it will do no harm to review it here.

Masson believes with Soldan ³ that the pigmented nevi are nervous, not dermal tumors; their type cell, according to him, is that of the sheath of Schwann. It can differentiate in various directions: in the superficial portions of the tumor beneath the epidermis it takes on a cellular, more or less epithelioid form, lying in nests that resemble Meissner corpuscles on the one hand and the smaller groups

* Received for publication December 15, 1931.

they were not. It remained to continue with the work, using more perfect and diverse methods of silver impregnation and any other procedure that might afford a clue as to their true nature. Accordingly, a method of impregnating the finest branches and end twigs of the fibers in thin, paraffin sections was worked out after considerable experimentation. This is detailed in another paper to which the reader is referred (Foot and Foot ⁴).

TECHNIQUE

Two variants of the silver method just mentioned (which will hereinafter be called the "p. g. s." method for sake of brevity) were used chiefly in the examination of a number of pigmented nevi of types which varied in respect to situation, melanin content, malignancy or non-malignancy, and so on. These were Variants 2 and 5; other variants sometimes proved themselves to be helpful. Various stains were employed to supplement these, and the Ranson-Cajal and Bielschowsky reduced silver impregnations, both slightly modified, were resorted to in order to impregnate the neurofibrils more specifically. It was found that the methods of mass impregnation which depend upon the use of silver diammino hydroxid tend to deposit too much silver in the epidermal layers and to underimpregnate the deeper ones, unless great care is exercised. The very portion of the block that one most particularly desires to examine seems to be the one in which there is the least detail and densest precipitate.

Ranson-Ramon y Cajal Method: Tissue blocks are fixed in absolute alcohol with 1 per cent ammonium hydroxid for 48 hours; they are then rinsed for $\frac{1}{2}$ to 3 minutes in distilled water, depending upon the size of the blocks. After being transferred to pure pyridin for 24 hours they are washed in many changes of distilled water for another 24 hours and then set in the dark in a 2 per cent solution of silver nitrate in distilled water at 37° C for 3 days. After rinsing in distilled water they are placed in a 4 per cent solution of pyrogallol in 5 per cent neutral formalin, made up with distilled water, for 1 to 2 days, after which they are rinsed in water and embedded in the usual manner in paraffin. The sections are a pale canary yellow, the neurofibrils are black, the endoneurium yellow and the myelinated fibrils old-gold, surrounded by a color-

ture of the two types, with a transition zone in between. This is no uncommon picture. He demonstrates nerve trunks, sometimes quasinormal, sometimes distorted by the proliferation of their Schwann cells, entering the tumors at their bases and becoming intricately involved in the cell complexes more superficially located. They then emerge from these to continue to a point beneath the epidermis, where they branch out into the subepidermal plexus. He also notes the fact that erector pili muscles are often directly involved in the tumor growth, sometimes appearing to share in it; this he explains on the basis that they develop from the same primordium. He carries these theories a step further and applies them to malignant nevi, "melanosarcomas" and "melanocarcinomas," as well.

In my previous article referring to results obtained with frozen sections impregnated with silver, no special mention was made of the tumor cells other than to point out the utter dissimilarity between them and the true epithelial elements of the epidermis. The paper concerned itself chiefly with the fibrils that were so strikingly demonstrated in silver impregnations, structures that Masson interpreted as collagenous fibers. That they are not ordinary connective tissue fibers, but rather endoneurium or actually nerve filaments, was indicated. It was shown that they were only imperfectly impregnated if the usual potassium permanganate-oxalic acid bleach was used before impregnating, whereas the reticulum and collagen of the stroma were, on the other hand, well silvered. Preliminary bromuration of the sections (calculated unfailingly to demonstrate astrocytes and neuroglia fibrils) produced much the same effects — the fibrils of the nevus nests were not impregnated, although the reticulum of the stroma was brought out sharply. It was seen that the finer fibrils of the nevi differed from those of a fibrosarcoma which were indubitably reticulum or collagen, and no such structures were demonstrable in sections from an epidermoid carcinoma, indicating that they were not of epidermal origin in the nevus or they would be present in the carcinoma also. Other stains failed to demonstrate them specifically; they were not fibroglia, neuroglia or elastic tissue.

There was, therefore, nothing definite to show just what these fibrils were, although several methods showed quite plainly what

Cytoplasm: When studied by ordinary methods the appearance of the cytoplasm is quite misleading; the epithelioid character of the cells, as noted in hematoxylin-eosin sections, is too familiar to need discussion. With silver methods their true morphology is brought out, and Masson's schematic plan is confirmed in every particular; the type cell is polygonal in its primitive form (Fig. 1), with short, hair-like processes. The coarser, tail-like processes are seen better in reduced silver sections where the cytoplasm impregnates more deeply than the nuclei (Fig. 2). They may also be demonstrated in the "p. g. s." method if Bouin's fixative is used instead of formalin, in which case the impregnation is reversed from a "positive" to a "negative" picture, as in the case of the Laidlaw method⁵ after Bouin fixation.

The more differentiated cells become, in this way, racquet-shaped; they develop one or two stout cytoplasmic "tails" and, by continuing the process, pass through a fusiform phase into enormously elongated, filamentous structures. Specimens impregnated with Variant 6 of the "p. g. s." method often bring out a web-like, filamentous, intracellular reticulum that may be concerned in the production of the nerve sheath webs under normal conditions. The fibrils of these will be discussed further later on. In the deeper layers of the tumors the scale-like, laminated form of type cell is found in Masson's "lames foliacées" or "leaf-like lamina" (Figs. 3 and 4). In malignant melanomas one sees bizarre distortions of these type cells (Fig. 5) bearing the same relation to them as the gigantic cells of glioblastoma multiforme bear to the glioblasts (spongioblasts), or those of fibrosarcoma to fibroblasts — they are enlarged caricatures of the type cells of the benign tumors. In fact, the nevus cell, when impregnated with silver, bears a striking resemblance to a neuroglia cell although, as we have seen in a previous paper (Foot and Zeek⁶), it does not impregnate specifically in bromuration methods which unfailingly demonstrate astrocytes in frozen sections.

The Laidlaw impregnation fails to demonstrate the nevus cells satisfactorily in sections from benign nevi fixed in formalin, Bouin's or Zenker's fluids; they are at best faintly brought out and are, therefore, "Laidlaw-negative," in comparison with the epithelium of the epidermis and its adnexae, which are positive and well shown.

less sheath. This method was very slightly modified by adding the Laidlaw oxalic acid-gold toning procedure. The deparaffinized sections were toned in 1:500 gold chlorid for 5 minutes and redeveloped in 5 per cent oxalic acid for a like period, washing between steps with tap-water; they were then fixed in 5 per cent sodium thiosulphate and washed well in water, after which they were mounted in the usual way in Canada balsam.

Bielschowsky Method: The blocks are fixed in 10 per cent neutral formalin, washed and treated for 3 to 4 days with pure pyridin. They are then washed in running water until there is no more odor of pyridin, and further washed in a few changes of distilled water to remove any chlorides. They are transferred from this to a 3 per cent solution of silver nitrate in distilled water for 3 to 5 days in the dark at 37° C, after which they are rinsed in distilled water and placed in a solution of silver diammino hydroxid in the dark at 37° C for 24 hours. This solution is made up by adding strong ammonia dropwise to 10 cc. of a solution of silver nitrate in distilled water of 10.2 per cent strength, until the resulting precipitate is just dissolved; 10 cc. of 3.2 per cent pure sodium hydroxid in distilled water is then added and the resulting reprecipitation again just dissolved with ammonia. The solution is then made up to 100 cc. with distilled water. The blocks are next washed in several changes of distilled water for 2 hours and then placed in 20 per cent neutral formalin in distilled water for several hours. After washing in water, they are then embedded in paraffin in the usual way. They are toned in gold chlorid intensified in oxalic acid, fixed in sodium thiosulphate (as above described) and mounted in Canada balsam. Further experiments with the Bielschowsky method, calculated to decrease its intensity without lessening its penetration, are now under way, but they are not wholly satisfactory in their present form.

The Ranson-Cajal method gives the most uniform results, but it is also apt to be somewhat uneven in its results.

CELLULAR MORPHOLOGY

Nuclei: These are rather vesicular and may contain one or more nucleoli which are more prominent in the malignant than in the non-malignant members of the group.

roughly parallel, chestnut-brown fibers that are intimately associated with the distorted sheaths formed by the proliferating tumor cells (Fig. 7). Actual connections between nerve trunks and subepidermal nests have admittedly not been traced in the form of continuous fibers which emerge from nerve trunks at the base of the tumor, continue through its intermediate cell complexes and terminate in or near the subepidermal nests; that this could be done in serial sections, given the requisite material and patience, is definitely indicated. The actual terminal distribution of the fibers is very difficult to determine; they seem to skirt the tumor cells, impinging upon them very closely, and to enter a complicated and incompletely impregnated rete in the pars papillaris of the skin. The besetting difficulty in all this work is that of determining the identity of nerve fibers, as distinguished from reticulum or collagen. All these usually stain or impregnate almost identically alike, so that it is only in the reduced silver sections that one may be at all sure of one's ground. Sometimes one may find sprouts of proliferating Schwann cells following nerve twigs into the subepidermal stroma, and in these cases, in reduced silver preparations, one may observe very delicate but intensely impregnated fibrils accompanying the cell columns and, apparently, not extending very much in advance of them.

That Masson's conception of an arborial distribution of the tumor along nerve trunks and branches is correct is everywhere indicated. If one should make tangential sections of small nevi, one may obtain some of them at levels that show mostly connective tissue of the corium; in this tissue the nerves will have been cut transversely and one will find that they are surrounded by nevus cells (Fig. 6), while these are to be seen nowhere else in the section. This is good proof that the tumor growth follows the nerve sheaths, but it does not tell us whether it is following them from below upward, or *vice versa*. Such pictures as that shown in Fig. 8, however, make it seem more probable that the former assumption is the more probable.

SUMMARY AND CLASSIFICATION OF FIBRILS

Collagen and Reticulum Fibrils: These are found chiefly in the stroma of the tumors, but there are many of them that bear a much closer resemblance to the endoneurial varieties of connective tissue

A strange fact is noted in this connection: the cells of malignant melanomas examined in the course of this work are usually "Laidlaw-positive." Just what this signifies is not yet evident.

The arrangement of the type cells follows Masson's description so closely that it would be futile to describe it again; his observations may be checked up in every particular. As to their melanin content, here, too, his observations may be confirmed. Some of the cells contain considerable quantities of melanin, while the bulk of their fellows are free from it. The presence of the pigment makes the racquet-like morphology of the melanoblasts more evident in those stains where the cytoplasm is not completely demonstrated, so that one might imagine that they differed from their fellows, were one to judge them by hematoxylin-eosin standards alone; when reduced silver is used, however, it becomes evident that the only observable difference between the two types is the presence of melanin in the one and its absence from the other.

FIBRILLAR MORPHOLOGY

With such stains as the Mallory phosphotungstic acid hematoxylin, the Van Gieson stain, and so on, the fibrils appear to be ordinary collagen. Using Variant 5 of the "p. g. s." method this is also true, for it does not differentiate between collagen and reticulum; if Variant 2 be used, however, many of the fibrils come out black like the reticulum. We have seen in the two preceding papers that reliable reticulum impregnations depending upon Mallory's bleach fail to demonstrate fibrils much beyond the limits of the stroma; those that are brought out in the nests by the "p. g. s." method fail to materialize satisfactorily if the bleach has been used. Therefore, one would appear to be justified in believing that these fibrils differ in some way from connective tissue collagen or reticulum. The reduced silver methods tend to obliterate the intercellular fibrillary details and to make the fibers appear like a homogeneous matrix (*cf.* Fig. 2), but they demonstrate nerve fibrils in the trunks and the cell nests. Such neurofibrils may be theoretically traced from the nerves at the base of the tumors, through the laminated sheaths to the subepidermal, epithelioid nests. When medullated they run as pale canals with a central axone through the laminated sheath expansions; when non-medullated they appear as curving,

course of nerves not as yet involved in a neighboring nevus, as well as by such pictures as the one shown in Fig. 3 where the nerve is entering an obvious tumor complex. The proliferation of Schwann cells along neighboring nerves could not be illustrated in this article by reason of space demands, but excellent photomicrographs in my possession attest to the veracity of the above statement.

An examination of malignant representatives of this tumor group reveals characteristics that one might be led to expect in malignant transformations: the cells become distorted, enlarged, lawlessly arranged, poorly differentiated, and their Laidlaw reaction becomes positive instead of negative; their fibrils no longer show the excellent endoneurial type of differentiation seen in the non-malignant tumors, but tend to be finer, less similar to true nerve sheath fibrils and often predominatingly intracellular.

The importance of the melanoblast as a type cell, in the case both of malignant and non-malignant "melanomas," dwindles as one examines these; some tumors may be almost exclusively composed of melanoblasts, but many will show very few. Masson's idea that they constitute nevus cells with a predominatingly chromogenic metabolism is everywhere strongly indicated, and the unfailing presence of cells, or groups of cells among them that show no demonstrable melanin and conform to the amelanotic type nevus cell, indicate that these are the more primitive, and therefore the true type cells of the tumor, while the melanoblasts are derived from them, rather than *vice versa*.

SUMMARY

Masson's theories that nevus cells are (1) derived from those of the sheath of Schwann, (2) non-pigmented in their pristine state, (3) associated with nerve trunks and fibers, and (4) closely related to those of the neurofibroma, are uniformly confirmed by an examination of a series of pigmented nevi by means of silver impregnations of several types.

than they do to ordinary connective tissue. Their caliber is very variable, not uniform; they are often recurved upon themselves, forming sharp angles and triangular varicosities at the point of reflection, and their staining properties are in every way similar to those of endoneurium. They do not form coarse collagen bundles, as does the connective tissue, and they do not resemble the reticulum of the areolar tissue or the lymphoid tissue. In the case of the laminated sheath swellings the fibrils are of the utmost delicacy.

Neurofibrils: These have already been described at length.

Protoplasmic Fibrils: These are found *within* cell bodies; in the non-malignant tumors they are in the cell processes, where they constitute a very delicate intracellular reticulum or "web"; in the malignant forms they may run all through the cytoplasm of the cells. The attenuated cellular processes, which Masson calls "nevus fibers," scarcely merit this term and should be recognized as filamentous extensions of the cytoplasm; it should be recognized, however, that they may contain the intracellular fibrillar webs just referred to, which are well shown in the case of Schwann cells in the sheath of a normal lingual nerve. A comparison of Figs. 2, 9 and 10 will make this clear. The above exposition probably explains the reason why more fibrils are demonstrable with the "p. g. s." technique than in the case of reticulum impregnations depending upon a preliminary bleach; the group of finest fibrils in the nevus nests are probably, for the most part, of a nervous or protoplasmic rather than of a reticular or collagenous nature.

DISCUSSION

It is, then, possible to confirm Masson's ideas regarding the histology of pigmented nevi and melanoblastomas in every particular; that the control has been made exclusively by means of silver methods that differ essentially from his trichrome stain, makes the confirmation the more striking. His conception of the morphology and distribution of the type cell, of its differentiation from a polygonal unit, through a fusiform phase to a filamentous, or (also) a flattened one, is completely borne out. His thesis as to the type cell's derivation from that of the sheath of Schwann is reinforced by the striking resemblance of the two in properly impregnated silver sections, and by the possibility of demonstrating columns of tumor cells along the

DESCRIPTION OF PLATES

The photomicrographs were made by Prof. Joseph B. Homan, with the assistance of the author.

PLATE 51

- FIG. 1. Field from a subepidermal nevus nest, showing the large Meissner type cells at the top and the smaller Merkel-Ranvier type at the bottom. "P. g. s." technique, Variant 2. $\times 800$.
- FIG. 2. A similar field, to demonstrate the morphology of the cells as brought out by the Ranson-Cajal reduced silver method plus gold toning. Note the pale nuclei and dark cytoplasm. $\times 800$.
- FIG. 3. One of Masson's "lames foliacées," or laminated sheath expansions impregnated to show its fibrillary endoneurium by the "p. g. s." technique. Variant 2. $\times 800$.
- FIG. 4. A similar field impregnated by the Bielschowsky reduced silver method in the block, with subsequent sectioning and gold toning. This shows the neurofibrils as well as the endoneurial fibers. The former may be seen entering the expansion at its lower pole and coursing among its cells. $\times 950$.

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PLATE 52

- FIG. 5. Distorted forms of nevus cells in a malignant melanoma shown in an edematous, inflamed portion of the tumor where their morphology is more evident on account of their spacing. "P. g. s." technique, Variant 2. $\times 800$.
- FIG. 6. A nerve trunk in transverse section penetrating a nevus nest in the derma. The axis cylinders are clearly visible. Below it is one of the distorted pseudo-Meissner corpuscles so often observed in pigmented nevi. A large axone (black rectangle) runs obliquely through it, surrounded by its clear sheath. Bielschowsky reduced silver method with gold toning. $\times 950$.
- FIG. 7. Longitudinal section of a nerve trunk running through a nevus nest. Many of its fibers appear to be non-medullated. Ranson-Cajal reduced silver method with gold toning. $\times 800$.
- FIG. 8. Topographic picture of a nerve of the corium entering a nevus at its base; just below center the nevus cells are sharply focussed, rather out of focus at the bottom. The nerve runs along the right border of the picture directly into the nests. The black masses at the upper left are collagen bundles in the corium. The section was cut tangentially with the epidermis and a millimeter or so beneath it, which demonstrates the intimate association of the peripheral nerves with the tumor which dips down along them from the upper, into the deeper skin layers. Bielschowsky reduced silver method, gold toning. $\times 350$.



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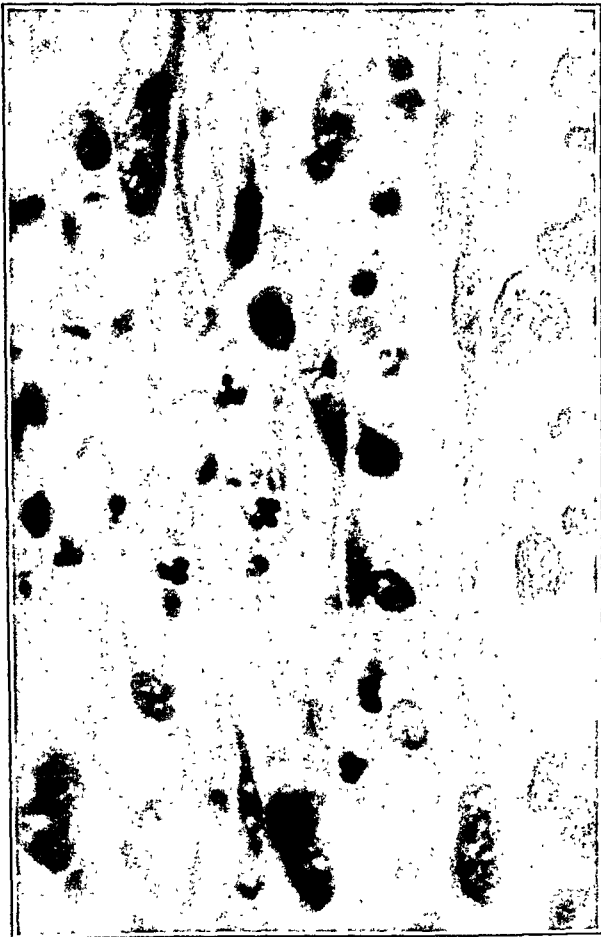


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PLATE 53

FIG. 9. A field to show the intermediate type of growth lying between the nevus nests and the "lames foliacées." Note the two types of cell and the intricate endoneurial fibrils. "P. g. s." technique, Variant 2. $\times 800$.

FIG. 10. For comparison with Figs. 2 and 9; a field from a nerve in normal human tongue impregnated by a Variant 6 of the "p. g. s." method. The striking similarity of the sheath cells to the nevus cells is at once apparent. The endoneurial fibers are not as sharply brought out by this variant as in that of Variant 2 or 5, but the cells and their connection with the sheath webs is so well shown as to make up for this. $\times 950$.



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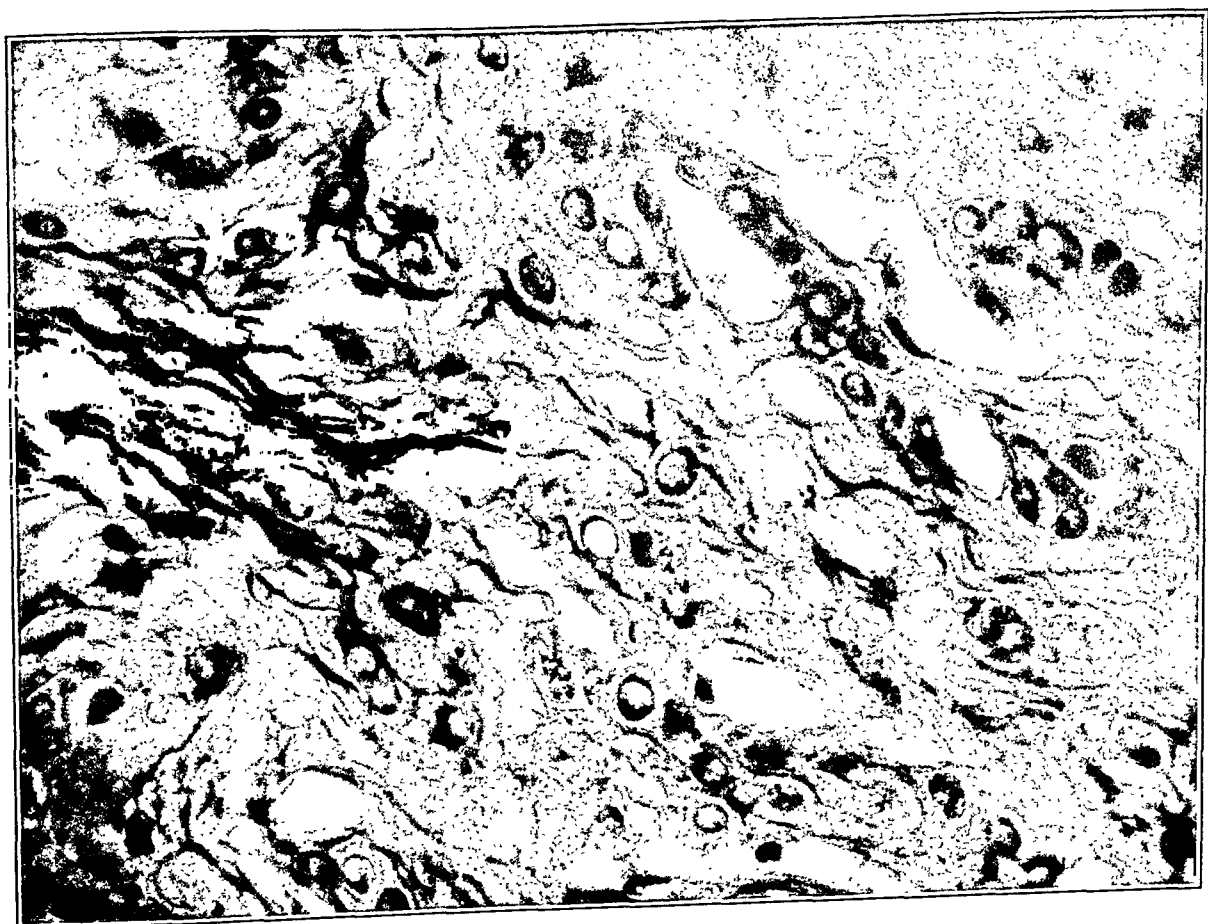
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TECHNIQUE

Rogers' method was followed very closely in the main, with a few slight changes in the procedure calculated to fit the case in point. In carrying out this technique it is well to wear rubber gloves, for the silver solutions are very strong and stain the fingers and hands intensely. A strong solution of potassium cyanide in water will remove these stains fairly well if used immediately, but its very poisonous nature makes it a poor and dangerous substance to have about the laboratory, and it should be employed with extreme caution.

Fixation: 10 per cent formalin is used, with a 2 per cent solution of ammonia in absolute alcohol as an alternative. Rogers also uses Bouin's fixative, and notes that either neutral or non-neutralized formalin may be employed.

Embedding: This is carried out in the usual manner, the blocks being dehydrated with 80 per cent, 95 per cent and absolute alcohol, but to each of these 2 per cent of ammonia is added. Then the blocks are kept for an hour or so in pure absolute alcohol, run through chloroform and chloroform-paraffin, and then into paraffin. After sectioning, the paraffin is removed with xylol, the sections run through absolute alcohol, and they are then kept for 12 to 24 hours in 95 per cent alcohol with 2 per cent ammonia. It was found that material embedded in the usual way, without the addition of ammonia, gave good results if the long, ammonia-alcohol treatment was resorted to after the paraffin had been removed. In fact, many of the illustrations in this article were made from sections so treated.

Impregnation: After a rinse in 80 per cent alcohol, the sections are placed in 40 per cent aqueous silver nitrate solution in the incubator for 20 minutes at 37° C, the staining-box being placed on the heating plate. This is a very concentrated solution, to be sure, but its use seems to be unavoidable. The sections are rinsed briefly in distilled water and treated with 20 per cent formalin for 5 minutes, after which they are changed into 1 per cent formalin for a few minutes. From this they are taken one by one, blotted briefly with filter paper to remove the excess formalin (but not to the point of dryness) and flooded with a few drops of a diammoniacal silver solution from a dropping-bottle. The solution is left on for 30 to 60 seconds, poured back into the bottle and the slides blotted off on

CONCERNING THE HISTOLOGY OF MELANOMA *

II. WITH SPECIAL CONSIDERATION AS TO THE NERVOUS ELEMENTS OF THE TUMOR

NATHAN CHANDLER FOOT, M.D.

*(From the Department of Pathology, College of Medicine, University of Cincinnati
and Cincinnati General Hospital, Cincinnati, Ohio)*

In the first paper under this title (Foot¹) much evidence was advanced in support of Masson's² theory as to the nervous origin of melanoma, but there were a number of important points that remained obscure and required further working out, chief among them the fate of the nerve trunks that were found traversing the tumor nests, a matter that was left largely to conjecture, the supposition being that they continued out to the epidermis and broke up to join in the formation of a more or less hypothetical nervous rete in the pars papillaris. One was left with the impression that the lack of a method that would definitely and metachromatically distinguish between nerves and connective tissue fibers and would demonstrate the ultimate distribution of the former, was a distinct handicap.

Rogers'³ technique for impregnating nerves in paraffin sections has supplied this deficiency in part, but its use has not entirely cleared up the matter, for the extremely delicate network of fibrils that surrounds the more primitive cells in the tumor nests still eludes definite classification. By applying this method, however, it has been possible to carry the investigation several steps further along its original line, reinforcing what has already been said and warranting the publication of a report on the progress thus accomplished.

As material for this report a number of benign and malignant melanomas were utilized. The benign tumors were of two distinct types: (a) those that were flat, brown and hairy, and (b) those that were more or less pedunculated and often almost non-pigmented. The malignant tumors were in part primary, in part metastatic. As normal control material, sections from a clavus of the toe and from the tip of normal human tongue were used. A small neurofibroma of the palmar surface of a finger served as additional control material and afforded interesting comparison with the melanomas.

* Received for publication March 14, 1932.

experiment with this step until he has obtained the desired results. Dilution, a little more or a little less ammonia, or variations in the length of immersion of the sections in the fluid may all be resorted to. The method will give brilliant results if one but perseveres in its use.

RESULTS OF THE INVESTIGATION

As tactile corpuscles and nerves play the leading part in Masson's theory and as we are interested in proving or disproving this, sections of tongue, of a corn and of a neuroma were first examined to determine the value of this method. The results are shown in Figs. 1, 2, 3, 4 and 5.

In the tactile corpuscles of the skin, the nerves run in medullary sheaths to the base of the organ, lose their sheaths and appear to ramify within the capsule among the epithelioid cells, apparently terminating in extremely complicated networks (Dogiel's "Retikolaren") see Fig. 2, or in club-like expansions (Fig. 3). In the tongue the latter are more usual and one finds, in addition, bodies resembling Grandry's corpuscles (Fig. 3), composed of two cells with a nerve filament lying between their apposed surfaces. The clubbed terminals may, in some instances, lie in the connective tissue outside of the tactile corpuscles. In both skin and tongue, heavy, webbed, non-medullated fibers may be found running about in the pars papillaris (Fig. 4). These are important structures to bear in mind later on, when the same fibers are being described in the case of the tumors. As to the neuroma (Fig. 5), it is found to be made up of coiled, parallel nerve trunks with medullary sheaths and an excess of fibrous tissue between trunks. The nerves seldom tend to stray from the neural sheath and the picture is, therefore, quite different from that to be described in connection with the melanomas.

Having established some standards, let us see what is found in the pigmented nevi. The large, flat variety shows numerous acini of primitive, polygonal cells that were described in the first paper; the larger, more epithelioid "Meissner" cells are not well represented. In such tumors it is possible to demonstrate nerve trunks at the base of the growths and trunks lying within their thin, perineural sheaths in the center of tumor nests (Figs. 6, 7 and 8). Often the nerves seem to impinge upon a tumor alveolus and stop at its margin (Fig. 7), but it is occasionally possible to demonstrate nerve fibrils apparently

the same filter paper. The reagent is made up as follows: to a given quantity (say 20 cc.) of 20 per cent aqueous silver nitrate, strong ammonia is added drop by drop until the resulting precipitate is just dissolved; then one drop of ammonia for each 2 cc. of silver solution (in this case 10 drops) is added to afford the necessary excess, after which the solution is diluted with an amount of distilled water equal to the original quantity of silver nitrate solution (here, 20 cc.).

The blotted sections are next placed in 20 per cent formalin, where they turn bright yellow to old-gold. It should be noted that *the sections should not be washed between steps, merely blotted long enough to remove the excess reagent.* It should also be remarked that no sodium hydroxid is added to the impregnating solution, as in the case of some other methods of impregnation.

Toning: The sections are then washed in distilled water and toned for 15 minutes in a 1:300 aqueous gold chlorid solution to which 2 cc. of glacial acetic acid has been added for each 100 cc. This is said to restrain the impregnation of the connective tissue fibers without interfering with that of the nerves, thus affording better contrast. It turns the sections from old-gold to grayish, or slate. They are then intensified by a 5 minute immersion in a solution containing 2 per cent oxalic acid and 1 per cent formalin. This changes the gray color to purple and gives more colorful pictures. After washing at the tap, fixing for 5 minutes in 5 per cent sodium thiosulphate and washing once more, the sections are run up through ascending percentages of alcohol into xylol and thus to Canada balsam, in the usual way.

The connective tissue is lavender to purple, the nuclei black, the nerves highly refractile and black to brownish black, and the only source of confusion is the impregnation of the fibrils of the erector pili muscles and the delicate fibers in the tumor nests; the reticulum is poorly impregnated throughout. All efforts to improve upon this method by reducing the strength of the very concentrated solutions utterly failed. There is only one reason for attempting improvements and that is the somewhat variable results obtained during the flooding of the sections with diammoniacal silver solution. It is supposed to be heated to about 50° C, but it was found to be too powerful when heated and the melanoma sections were overimpregnated as a result. The investigator should

muscles with the tumor cells (Fig. 12). In almost every tumor examined these were either closely surrounded by tumor cells or they were broken up so that small, atypical smooth muscle cells became intercalated between tumor cells. Masson has already stressed this close association. In one tumor strands of skeletal muscle are found near sebaceous glands, running fanwise into the tumor. Both types of muscle cell, however, appear always to be in the minority and may be merely "sympathetically" involved, as is often the case with basal cells of the epidermis over such tumors. If one attempts to revamp the scheme of the pigmented nevus on a muscular basis one does not get very far, but that we must bear in mind the possibility of this being a mixed tumor is clearly indicated.

It seems, then, that benign pigmented nevi fall into two extreme types: those poor in nervous elements and composed of primitive, polygonal cells in ovoid nests (grossly, flat tumors) and those rather rich in nervous elements and composed largely of what appear to be perverted Meissner corpuscles, in the form of Masson's "*lames foliacées*." Transitions between the two types are so frequent that it seems unwise to attempt a definite separation of these — they are described merely for the sake of accuracy.

The malignant tumors examined did not throw much light upon the subject — they get too far away from the rather complicated differentiation of their benign congenors for one to draw very definite conclusions concerning them. When they metastasize to lymph nodes they are even less obviously related to the benign type, for they are quite innocent of pseudo-Meissner bodies and nerve filaments. In their primary sites, however, they possess more or less similarity to the benign growths and there seems to be no reason for our recasting our theories as to the relationship of the two.

Masson derives the type cell of the tumor from that of the sheath of Schwann and this idea was adhered to in my first paper, but now that a good method for staining the nerve fibers has been found, one hesitates whether to ascribe the origin of the type cell to the Schwann cell or to those of the endoneurium and perineurium. It is very difficult to decide the question on the basis of the material at hand. Comparing melanomas with a neuroma, however, one may say that the latter consists chiefly of nerve trunks with proliferated epineural or fibrous sheath cells and interneural fibrous tissue; the

running into an alveolus (Fig. 8). All attempts to connect them definitely with the fine reticulum among the tumor cells failed.

The second type of mole examined, more or less pedunculated and definitely raised above the epidermal surface, shows more cells of the epithelioid type and much more resemblance to Meissner corpuscles. In some cases the nerves appear to run in the interacinar stroma, to terminate either in blunt coils or in branching, finger-like terminals with something of the appearance of abortive Dogiel endings (Fig. 9). In other instances one finds large, clubbed cells here and there that resemble the nerve terminals in the Meissner bodies, but are unconnected with nerve trunks. One also notes, among these, double groups of cells resembling Grandry bodies, but again, lacking nerve filaments. In this type of tumor it is possible to trace the medullated nerve trunks much farther out toward the epidermis than one can in the preceding type. Many of the trunks almost immediately become non-medullated and of the thick, webbed type shown in the pars papillaris of normal controls. These may show very fine forms with varicosities along their course, lying among tumor cells and coursing along their borders.

One rather large, sessile mole, that was already used in the work described in the preceding paper, shows very striking differences when compared with its fellows, with the exception of one mole that was very similar to it in its gross appearance. One is immediately struck with the presence of numerous elongated cells that impregnate deeply and are often reticulated, resembling the clubbed terminals of the nerves in the Meissner bodies. Among these one notes numerous stout fibers that impregnate in a peculiar manner and are somewhat similar to those seen in the pars papillaris of the skin; they contain vacuoles at intervals and are otherwise more compact than the webbed filaments already referred to (Fig. 10). It is at once evident, on examining the "lames foliacées" of this tumor (structures not prominent in the flat type of nevus) that these present most of the distinguishing features of Meissner bodies. The large, clubbed endings are often suggested and structures that appear to be abortive attempts at the formation of Dogiel's "Retikolaren" can be easily found (Fig. 11). In isolated instances, medullated fibers are seen traversing the laminated bodies in their long axis.

One is made increasingly uneasy, while studying all types of these benign tumors, by the very intimate association of the erector pili

DESCRIPTION OF PLATES

All the photomicrographs were taken from sections impregnated by the Rogers' technique. They are all at 800 diameters magnification, except Fig. 2, which is about 2000 diameters. They were made by Prof. J. B. Homan, of our Department of Medical Art, with the assistance of the author.

PLATE 54

- FIG. 1. Meissner corpuscles just beneath the epidermis of a clavius.
- FIG. 2. Oil immersion photomicrograph of a portion of a Meissner corpuscle, to show the complicated, webbed "Retikolaren" of Dogiel.
- FIG. 3. Two Meissner bodies from normal human tongue, showing a somewhat simpler form of nerve terminal than that seen in the preceding pictures from the skin. Note the numerous nerve filaments in the stroma and the two reniform nuclei with a small fibril between them (Grandry body?).
- FIG. 4. Reticulated non-medullated fibers from the pars papillaris of the epidermis, one fibril running across an epidermal papilla.

melanoma, on the other hand, appears to consist of cells connected more especially with the nerve terminals and their adnexa and, to a lesser extent, with the end filaments themselves. This bears out Masson's theory in every particular save one — the derivation of the type cell from the Schwann cell — and this cannot be refuted; it seems safer, for the present, not to be too categorical as to the derivation of the type cell, beyond saying that it is probably derived from one of the cells of the inner neural adnexa.

SUMMARY

By means of Rogers' technique of silver impregnation, it has been possible to demonstrate nerve fibers in melanomas and to show a striking resemblance between Masson's "lames foliacées" and the normal Meissner corpuscles, not only in respect to their morphology (which Masson has already brilliantly shown), but also in connection with the distribution of nerve filaments in and about them. This article merely reinforces what was said in its immediate predecessor and supplies some of the deficiencies that were to be noted in that paper, which were due to the lack of a suitable method for attacking the problem.

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PLATE 55

FIG. 5. Field from a small neuroma, to demonstrate the coarse medullated fibers of which it is largely composed, together with some finer fibrils. This was chiefly made up of fibers like those at the upper right and lower middle portion of the picture. Distortion of the large fibers is due to formalin fixation.

FIG. 6. A nerve trunk lying in a tumor nest; note the perineural sheath.

FIG. 7. A trunk with coarser fibrils impinging upon the base of a tumor nest, but not penetrating into it very deeply.

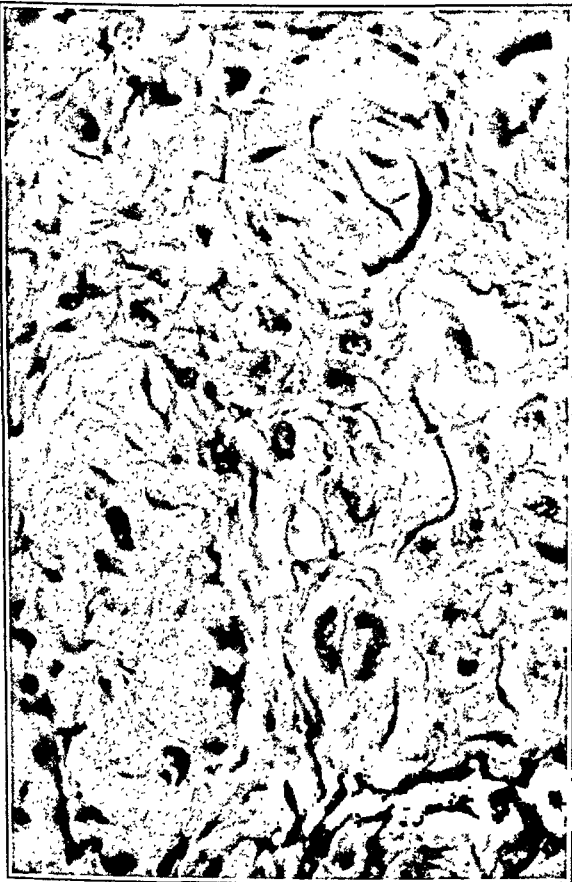
FIG. 8. A similar trunk leading into a small tumor alveolus; here, too, the fibrils appear to fail to penetrate deeply; one of them has a lancet-shaped terminal swelling, indicating that the fibrils do not communicate or connect with those among the cells.



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PLATE 56

FIG. 9. Coarse nerve bundle from a raised, sessile mole. It runs in the inter-alveolar stroma and apparently terminates in finger-like branches.

FIG. 10. Compact nerve fibrils, with small vacuoles, seen in the tumor tissue of a sessile, non-pigmented mole of the face.

FIG. 11. A "lame foliacée" from the tumor shown in Fig. 10, to be compared with Figs. 1, 2 and 3. A few structures resembling rudimentary Dogiel terminals may be seen and a body very much like an enormously elongated nucleus, but probably a nerve terminal, may be noted near the center of the field.

FIG. 12. Erector pili smooth muscle fibers intimately associated with the tumor shown in the preceding figures, coarse "nevus fibers" and nerve fibers are seen here and there.



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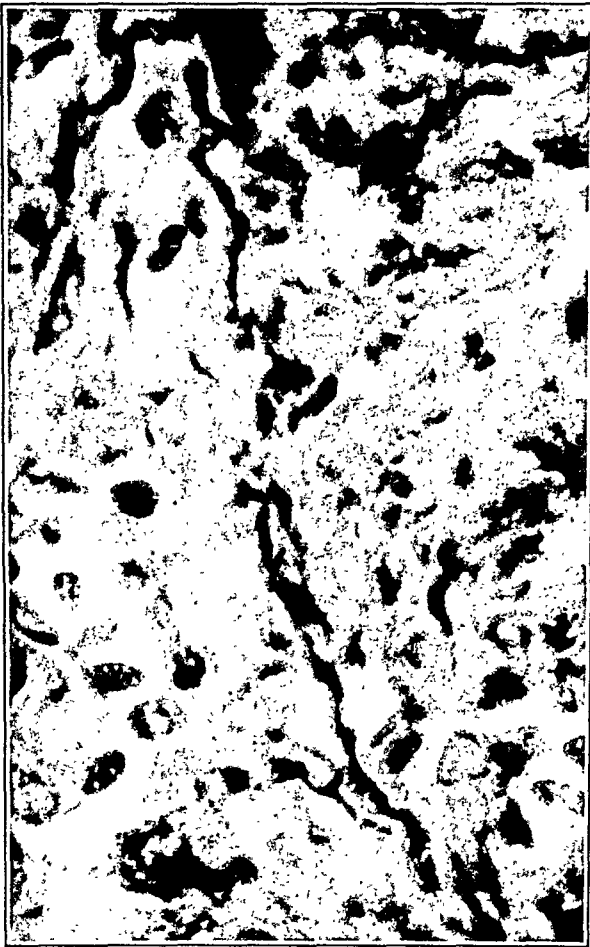
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The inorganic remains of the various cancerous growths were studied in dark-field illumination obtained by using a Zeiss cardioid condenser. The material was incinerated in an electric quartz oven at a temperature of 650° C in the manner previously described.⁴ Comparative examinations of all incinerated neoplastic material, with the accompanying control histological preparations, demonstrated how admirably the cancerous newgrowths lend themselves to this technique, as the fixed mineral elements retain their distinctive morphological organization.

The first preliminary observations of the microincinerated tumors confirmed the previous contentions of Policard and Doubrow that cancerous tissue remains carbonized longer than normal tissues. Another striking phenomenon we detected was that the intensity and distribution of the mineral ash deposit is more abundant within the invading newgrowths than in the surrounding stroma. This extraordinary concentration of the inorganic salts in the tumor tissue is well shown in the accompanying photomicrographs (Figs. 1, 3 and 4), and was found to be a feature peculiar to both human and rodent neoplasms.

More detailed analysis of the breast carcinomas shows that the infiltrating cancerous newgrowths are characterized by a heavy deposition of mineral salts when viewed at low magnifications. Observation with oil immersion lenses shows that the nuclei contain rather more ash residue than do nuclei of the normal duct tissue. This deposit is concentrated along the peripheral margins of the nuclei (Figs. 3 and 4) and corresponds well with the hyperchromatization described by Horning and Richardson⁵ in malignant growths. The nuclear inorganic salts contain visibly more iron oxide than do those of normal cells.

The remaining mineral deposits in the cytoplasm are more abundant than in the normal cells and contain an appreciable quantity of calcium and iron salts.

There are evidently at least three factors causing an increased appearance of ash in the cancerous ingrowths when viewed with the low power of the microscope. There are more nuclei per unit area present than in the adjacent fibrous stromal tissue (Figs. 3 and 4), and the nuclei themselves contain more inorganic residue than do those of the normal cells (Figs. 2 and 4). In addition to these factors the cytoplasm of the neoplastic cells contains more mineral salts than is usual for such tissue (Fig. 2).

HISTOCHEMICAL STUDIES BY MICROINCINERATION OF NORMAL AND NEOPLASTIC TISSUES *

GORDON H. SCOTT

AND

E. S. HORNING

ROCKEFELLER FOUNDATION FELLOW

*(From the Department of Anatomy, Washington University School of Medicine,
St. Louis, Mo., and the Departments of Anatomy and Cancer Research,
The University of Sydney, Sydney, Australia)*

Policard and Doubrow¹ in 1924 were the first investigators to apply the technique of microincineration to a comparative study of normal and malignant tissues. Apart from finding a slight difference in the mineral ash content between the cancerous and corresponding normal cells, they demonstrated most convincingly the advantages of this method, not only as a histochemical but also as a pathological technique. Recently Scott² devised an improved method by which the inorganic structure of the incinerated material can be more readily observed, and also by which the intensity of the mineral salt deposits is more appreciably recognized. With the recent use of this method the results obtained by Horning and Scott,³ which indicate that morphogenesis in the developing embryo is accompanied by apparent differentiation of the inorganic constituents, are extremely interesting when correlated with the Cohnheim "embryonal theory," according to which tumors are held to proliferate in much the same way that embryonic tissue grows. Under these circumstances it was considered advisable to reinvestigate the inorganic nature of malignant and normal tissues by means of this improved technique. The neoplastic material consisted of human medullary duct carcinomas of the breasts, as well as of several of the scirrhous types,† together with the following transplantable mice tumors: M 63, S 37 and S 180.

* Aided by an appropriation from a grant made by the Rockefeller Foundation to Washington University for research in science.

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† We desire to express our thanks to Dr. Robert Elman who supplied us with this material. Thanks are also due to Dr. Leo Loeb and Dr. F. Carter Wood for providing these rodent transplanted tumors.

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DESCRIPTION OF PLATES

PLATE 57

- FIG. 1. Photomicrograph of an incinerated section through a human duct carcinoma of the breast. Observe the difference between the mineral ash content in the infiltrating malignant tissue and that of the adjacent fibrous stromal structures.
- FIG. 2. Showing camera lucida drawing of the same, as seen under a higher magnification. Note the conspicuous peripheral accumulation of inorganic salts in the nuclei of the invading tumor tissue.

A comparative examination of the transplantable rodent carcinoma and the sarcomatous growths reveals that both are rich in mineral ash deposit. The differences, however, between the stroma and the infiltrating malignant growth are less marked than in the human neoplasms, as the cells composing the surrounding stromal regions contain more inorganic residue. Nevertheless, the distinction between the pathological and the adjoining healthy tissues is clearly defined. Another interesting feature is that the nuclei of the sarcoma cells of the mice appear to contain greater concentrations of inorganic material than do the nuclei of carcinoma M 63, while the mineral salts in the cytoplasm are distributed in a more diffuse manner.

A survey of the inorganic structure of neoplastic and normal tissues demonstrates conclusively that malignant growths are richer in their mineral contents than normal tissues — especially in calcium and iron oxide. Beebe and Clowes,⁶ employing biochemical methods, have shown that necrotic tumors contain more calcium oxide than rapidly growing cancers free from necrosis. Although necrotic areas incinerate less readily, our observations by microincineration yield supplementary evidence.

CONCLUSIONS

The results obtained from this investigation are of interest, inasmuch as they have demonstrated that functional differences between cancer and normal tissues are exhibited inorganically by marked variations in their inorganic content.

An additional feature is the close similarity between developing embryonic cells and cancer cells — a similarity which is mainly due to the distribution and arrangement of mineral salts. Both of these cells are characterized by an extraordinary variation in the intensity, concentration and orientation of their inorganic constituents, and contrast greatly, on the other hand, with the appearance of the mineral elements in the healthy adult tissue, which remain proportionally fixed. This “inorganic reversion” of the cancer cell, as revealed by microincineration, is interesting in view of Cohnheim’s theory to the effect that malignancy depends upon the retention of small groups of cells of embryonal character.

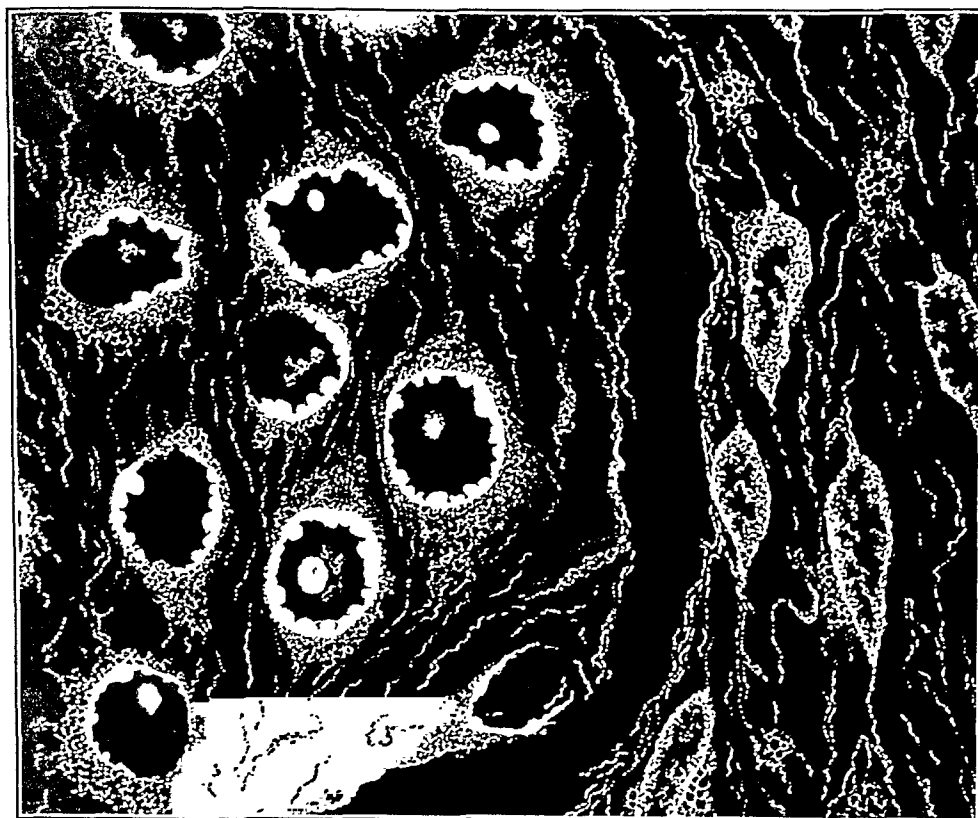
PLATE 58

FIG. 3. Photomicrograph taken with higher magnification, depicting incinerated sections of a human duct carcinoma of the breast. Observe the increased mineral salts in the invading growths and compare this with the inorganic remains of the surrounding stroma.

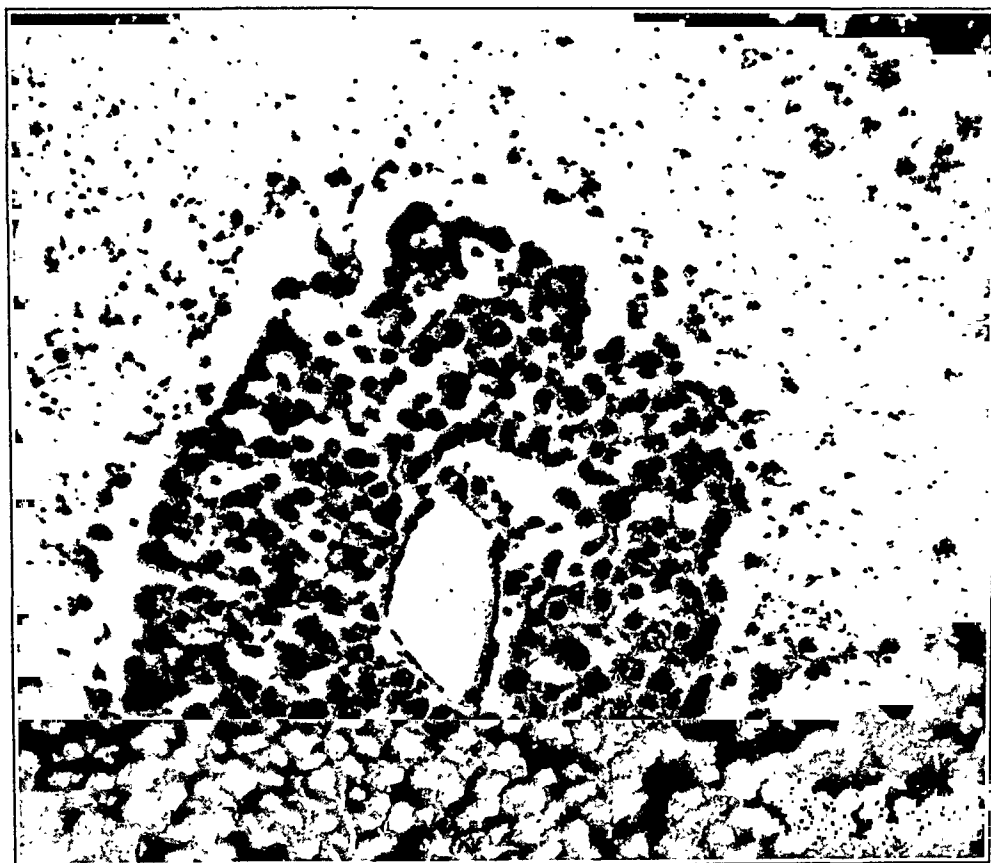
FIG. 4. Showing the curious peripheral concentration of mineral salts in the nuclei of the neoplastic tissue.



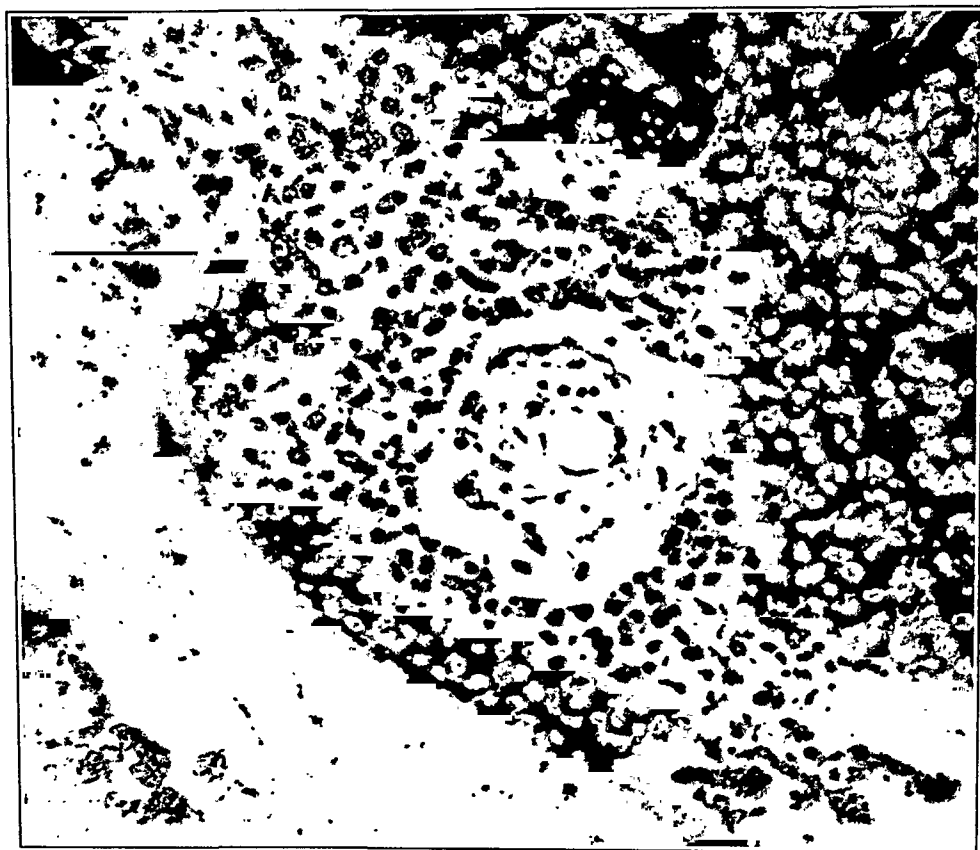
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The patient was readmitted to the hospital on June 7, 1926, and on the 19th, removal of the growth was done again with a similar application of radium. She was retained in the hospital under constant observation, but on August 17 she developed such marked stenosis that a tracheotomy was performed and the tube retained until her subsequent death.

Following the tracheotomy, at several intervals, large masses of papillomatous material were removed from the larynx. However, her physical condition was so improved by the tracheal operation that her father insisted upon taking her home. In spite of instructions that he was to bring her back at stated intervals for the cleaning of her tracheal tube, this was but rarely done.

In March 1927, the last removal of small papillomatous masses from the larynx was done. There seemed to have been a marked reduction in the general tendency to redevelopment. However, the following summer she was brought into the hospital in a dying condition by her father, he having attempted to change the tracheal tube and failing to reinsert it properly. In all, this child was in the suspension apparatus ten times.

An autopsy was performed and the following report of Dr. Oesterlin, describing unusual pathological findings, is here presented.

GROSS FINDINGS

Well developed, normal child, 3 years of age, with a tracheotomy wound and tube *in situ*. After removal of the cannula, a large papillary tumor was found projecting into the wound.

Upon opening the trachea, the whole of the larynx and the upper part of the trachea were found to be filled with cauliflower-like masses, the single elements of which presented a distinct papillary structure varying in size from that of a millet seed to that of a lentil. A few of these masses were larger and attained the size of peas. They were grayish white in color, and of firm consistence.

The larynx was entirely filled with these masses in such a way that no details of its structure could be made out. The epiglottis could hardly be seen and was almost entirely covered with papillary tumors.

The adjacent organs, the esophagus and the large blood vessels, were intact and there was no invasion of the tumor into them. The thyroid gland was small and showed no lesions.

A CASE OF MULTIPLE PAPILLOMATA OF THE LARYNX WITH AERIAL METASTASES TO LUNGS *

HENRY B. HITZ, M.D., AND ERNST OESTERLIN, M.D.

(From the Milwaukee Hospital, Milwaukee, Wis.)

Dorothy G., aged 2 years, white, female, was admitted to the Milwaukee Hospital, March 16, 1925, with difficulty in breathing and inability to speak aloud. The history, as obtained from her parents, was that at the age of 1 year she had a dry irritative cough followed by a gradual loss of voice, and since that time has been able only to whisper. For two months prior to her admission to the hospital she had occasionally at night a rather marked dyspnea. The child had been a full-term baby, normally delivered, was breast fed for the first thirteen months, and had previously not been ill. At the time of admission she was well developed and well nourished. There was nothing abnormal in the family history. The father and mother were strong and physically well, as were two other children, one older and one younger.

Examination at the time of admission showed no abnormality in the nose, nasopharynx or throat. The tonsils were small and normal in appearance and there was no perceptible adenoid mass present. The larynx showed what appeared to be multiple papillomata covering the vocal cords and much of the ventricular bands.

On the following day, March 17, under direct laryngoscopy, numerous papillomatous masses were removed from the larynx and sent to the laboratory for sectioning. The pathological diagnosis was benign papilloma.

After a short period of time in the hospital she was discharged, but was readmitted with similar symptoms, Sept. 9, 1925. Examination showed redevelopment of papillomata in the larynx, and they were removed again under direct laryngoscopy. She was discharged with improved breathing and speech, but a few months later, on March 20, 1926, she was readmitted and underwent similar treatment. On this occasion, while still in the suspension apparatus, she was given an application of radium for two and one-half milligram hours, and after a few days discharged.

* Received for publication December 8, 1931.

COMMENT

The interpretation of the primary growth is not difficult. There is little doubt that here we were dealing with multiple papillomata of the larynx. The growths were removed from different sites at different times and always showed the same type of growth as can be seen in Fig. 1.

The presence of these tumor masses in the lungs is not as easily understood. At first glance, on the postmortem table, it was thought they might be complicating tuberculosis, but under the microscope they are distinctly seen to be a neoplastic growth (Fig. 2). The question then arises whether or not these nodules in the lungs were metastases from the tumors in the larynx. The age of the patient, and the evidently benign type of growth, seem to speak against metastases. In order to detect and definitely rule out a metastatic dissemination of the tumor through lymph channels, many sections of the peritracheal and peribronchial glands were made without finding metastases in these lymphatic structures.

Another possibility is that the nodules in the lung had an origin analogous to the growth in the larynx, resulting in a condition of multiple papillomata in the bronchioli. In this case it must first be assumed that a metaplasia of the columnar epithelium of the bronchiolus took place into a squamous stratified epithelium, from which this type of tumor only can arise, but no reason was found to substantiate such a change. Furthermore it is difficult to understand why the lower half of the trachea and all of the larger bronchi should have been free and only the smaller bronchioli filled with tumor masses. It is also not easy to trace the connection of each nodule with the bronchiolus; sometimes the tumors were lying free in the lung alveoli.

The interesting findings in Figs. 3 and 4 suggest another interpretation. These slides show plainly how the tumor completely fills a part of the lumen of a bronchiolus. This would suggest the interpretation that all of these tumors in the lung had the same common origin, namely, that the tumor masses growing too rapidly in the larynx were detached and carried into the bronchi by aspiration. They passed the larger bronchi but were caught in the bronchioli, obstructing their lumina. In this way they became implantation metastases and began to grow into the alveoli. In some areas con-

The lower half of the trachea and the large bronchi showed no pathological findings. In the lungs, however, there were many small cavities, varying in size, some as large as hazel nuts. Frequently their connection with the smaller bronchioli could be traced. The walls of these cavities were covered with fine granules about the size of millet seeds. The other organs throughout the body were found to be normal.

MICROSCOPIC FINDINGS

The primary growth from the larynx and trachea (Fig. 1) presents a stalk of connective tissue, rich in hyperemic blood vessels. The epithelial lining of this stalk consists of many layers of squamous stratified epithelial cells without hornification. In the upper layers the cells are large and polyhedral. In the basal layer the cells are columnar and their nuclei stain more intensely with hematoxylin. The cells are regular throughout, without any remarkable difference in size or shape. There are a few polymorphonuclear leukocytes scattered among the epithelial cells. The submucosa is in some areas invaded by lymphocytes. As is usual in papilloma of the larynx there are many mitoses.

The nodules in the lungs (Fig. 2) sometimes form compact masses, but frequently contain central lumina which are either empty or filled with desquamated cells and polymorphonuclear leukocytes.

The tumor cell strands consist of squamous, stratified epithelial cells resembling those found in the larynx. There are large, polyhedral cells which stain lightly with hematoxylin. Columnar cells are frequent; they form not only the basal layers as in the primary growth, but are also to be found everywhere between the polyhedral cells. Mitotic figures are still more frequent than in the tissue from the larynx.

The strands of epithelial cells are in some areas directly adjoining the lung alveoli; in others there is a zone of connective tissue which separates the tumor cells from the walls of the alveoli.

A view of Fig. 3 shows a white space lined by columnar epithelium, which apparently corresponds to the epithelial lining of a bronchiolus. This lumen contains, besides detritus and white blood cells, some squamous stratified epithelial cells of exactly the same structure as were found in the papilloma of the larynx (Fig. 4).

SUMMARY

The case here described is one of multiple papillomata of the larynx with metastases to the lungs through the bronchi (aerial metastases).

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DESCRIPTION OF PLATE

PLATE 59

- FIG. 1. Primary growth. Papilloma of larynx.
- FIG. 2. Aerial metastases. Low power.
- FIG. 3. Bronchus showing aspirated tumor mass in its lumen.
- FIG. 4. Central part of lumen (papilloma under high power).

nective tissue had already been formed, apparently as a reaction against the foreign body, such as would result from stray particles of tumor tissue invading the lung.

DISCUSSION

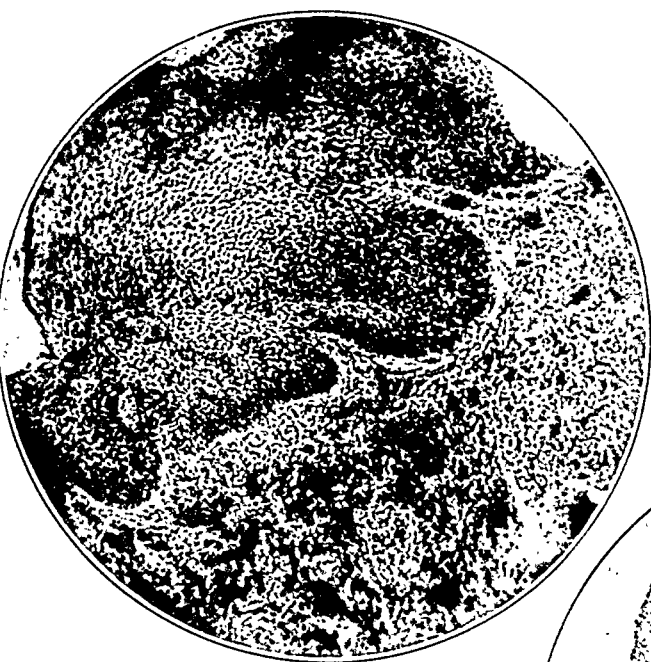
The text-books of pathology mention little or nothing about metastases through the bronchi. Only in the French literature is attention given to this possibility. Ribadeau-Dumas¹ mentions the "greffe bronchique" (bronchial graft) for aspirated particles of a cancer of the esophagus which become grafted in the parenchyma of the lung.

Letulle² describes them as uncommon, but very characteristic. He mentions as primary growths, epitheliomata of the pharynx, larynx and trachea. It is easily understood that pedunculated vegetations in the larynx may at intervals shed some fragments or isolated elements, which are still endowed with karyokinetic activity.

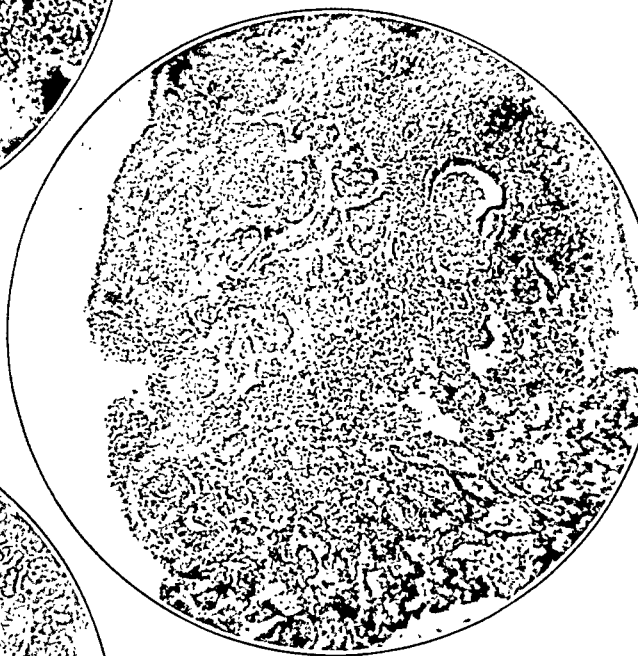
Letulle and Jacquelin³ have described a very interesting case of a collapsed lung in which a primary cancer developed. "From this primary growth neoplastic colonies arose in the normal bronchioli by 'aspiration.'" They were grafted and formed carcinomatous nodules. "Around the bronchus they grew, not only into the interior of the bronchiolus, but especially into the alveoli connected with this bronchiolus. This almost systematic disposition of the peribronchic alveoli by the cancer cells cuts out a circular, almost regular zone." Letulle has coined for this type of metastases the term, "metastases aeriennes" (aerial metastases).

In the case here presented, the tumor was histologically non-malignant, both in the larynx and in the lung. Nevertheless the tumor represented an actively growing, benign neoplasm, from which small fragments frequently became detached. It was possible to find these fragments in the center of a bronchiolus and to trace the origin of the tumor nodules in the alveoli from the invaded bronchioli. In spite of a careful search, no invasion of the lymphatics by tumor cells was observed.

All of these facts give definite evidence of the invasion of the lungs, through the bronchi, by a benign papilloma, primary in the larynx. If this be true, closer attention should be paid to the occurrence of this type of metastasis, with the probability that more cases of this type will be observed.



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father the child was born at term, and was apparently a normal baby except for a lump over the lower part of the back. There was no information available as to how large the mass was at birth, but the parents believed that it had gradually increased in size. There had been no other complaints. The child talked when 12 months' old and walked at 14 months. At times she indicated when she wished to micturate or defecate.

Examination: The child was very well developed and weighed 9420 gm. (20.7 pounds). There were palpable cervical glands. The head measured 47 cm., and the chest 47 cm. in circumference. The child walked without difficulty, and seemed quite normal except for the large, soft, tumor mass in the lumbosacral region (Fig. 1). The mass measured 10.5 by 9 by 4 cm., and was covered by healthy skin, upon the surface of which were two small, pedunculated nodules. There was no detectable abnormality of sensation. Reflexes were all present and equal.

X-ray Report: Roentgenograms of the entire vertebral column from the first thoracic vertebra downward revealed a marked defect of the posterior part of the neural arches of the fourth and fifth lumbar vertebrae and of the entire sacrum.

Laboratory Data: Examination of the urine revealed nothing abnormal. The blood Wassermann reaction and the Kahn test were negative. Numerous examinations of the blood revealed a leukocytosis of from 11,850 to 27,000. The percentage of polymorphonuclear (neutrophilic) leukocytes varied from 15 to 35, while mononuclear leukocytes ranged from 59 to 80 per cent. Definitely pathological cells could never be detected although many smears were examined. The red blood cells varied from 3,680,000 to 4,600,000 per cmm. The hemoglobin varied from 65 to 88 per cent by the Sahli method.

Operation: On August 6, 1931, the tumor was excised. A midline incision was made over the mass through the skin and a very thick layer of adipose tissue down to a small tumor nodule about 2 cm. in diameter. The adipose tissue was readily dissected off the mass, revealing a narrow stalk passing downward from the nodule through the defect in the posterior vertebral arch into the spinal canal. The stalk was cut through at its proximal end. The exact relation of the stalk to the spinal meninges and neural tissue was not determined. No operative repair of the spina bifida was attempted.

Postoperative Course: Following the operation the patient made an uneventful recovery and was discharged on September 13, 1931. Examination before discharge from the hospital did not reveal any abnormal, objective neurological findings. The etiology of the leukocytosis was never determined. Roentgenograms were made of all long bones, the chest and the gastro-intestinal tract; none of which revealed anything abnormal. The child was last seen on October 13, 1931, at which time she seemed in excellent health. A leukocytosis of 13,100 was still present. The red blood cells numbered 3,820,000 per cmm. and the hemoglobin was 76 per cent. The head has not enlarged.

Neoplasm: The tumor itself was fixed in formalin, embedded in paraffin, and stained with hematoxylin-eosin, mucicarmine, Van Gieson's acid picro-fuchsin, and Freeman's method for nerve fibers. On incising the tumor mass a small cyst, the wall of which was pedunculated and thrown into folds, was opened (Fig. 7). This cyst contained a thick, glary, tenacious, mucus-like fluid.

LUMBOSACRAL TERATOMA ASSOCIATED WITH SPINA BIFIDA OCCULTA *

REPORT OF A CASE WITH REVIEW OF THE LITERATURE

PAUL C. BUCY, M.D., AND H. E. HAYMOND, M.D.

(From the Department of Surgery of the University of Chicago, Chicago, Ill.)

Mixed tumors and teratomas of the sacral and sacrococcygeal regions have long been recognized and very frequently described in the literature. Ewing¹ reports: "They are bulky masses, present at birth, lying on the dorsal surface of the sacrum and coccyx, and adherent to or enclosed within the periosteum, or connected to the bone by a pedicle. Others lie anterior to the sacrum, connected with this bone, or with the rectum, and projecting into the pelvis. The structure presents cystic and solid portions similar to those of teratoid tumors, including cysts lined by various types of epithelium, dermoids, segments of intestinal mucosa, gland structures, fat, muscle, cartilage, and bone and finally, portions of nervous and glia tissue. In addition, a great variety of rudimentary organs are observed. These include segments of intestine with mesentery, rudimentary esophagus, stomach, and buccal cavity with salivary glands, pulmonary parenchyma, bronchi with cartilaginous rings, thyroid, pancreas, spleen, adrenal, kidney, brain with ventricles and choroid plexus. The bones may reproduce well-formed extremities, as forearm and hand, tibia, femur, and joint, pelvis and extremities, toes, and eyes. In fact, Askanazy regards the sacral teratomas as the most prolific in the production of rudimentary organs." On the other hand similar lesions of the lumbosacral region are exceedingly rare, and for this reason we present a case of lumbosacral teratoma associated with a lower lumbar and sacral spina bifida.

CASE REPORT

CLINICAL HISTORY: *Female, aged 15 months, mass in lumbosacral region since birth. Spina bifida. Unexplained leukocytosis. Extirpation of teratomatous tumor. Recovery.*

E. Del M., a Mexican female, aged 15 months, was admitted to the Bobs Roberts Memorial Hospital for Children on August 3, 1931. According to the

* Presented before the Chicago Pathological Society, February 8, 1932.

Received for publication February 29, 1932.

which there were many bundles of myelinated nerve fibers, a ganglion containing many ganglion cells, an atypical Vater-pacinian corpuscle, a lymph node and much smooth muscle. The myelinated fibers did not differ from similar bundles of fibers found elsewhere in the body (Fig. 6). The ganglion (Fig. 6) was comparable to those found normally in the sympathetic nervous system or to the posterior root ganglia. It contained many typical large ganglion cells. Unfortunately the various stains did not demonstrate the processes of these cells and it was impossible to state whether they were unipolar or multipolar cells. Each was surrounded by a group of typical spindle-shaped capsule cells and numerous nerve fibers could be seen running through the ganglion. The Vater-pacinian corpuscle (Fig. 4) was found in the tumor not far from the ganglion and amongst the bundles of myelinated nerve fibers. The corpuscle had a definite connective tissue capsule, beneath which were several layers of fine concentric rings associated with a few flat, elongated nuclei. This large ring of fibers enclosed five smaller but similar rings, the central portions of which contained numerous concentric rings of fine fibers but no nuclei. In one of these smaller rings a central canal comparable to the inner bulb of the normal corpuscle was seen. There was also within the larger ring a small collection of cells with large oval nuclei and no definite cytoplasm. The lymph node presented nothing remarkable. It was composed of numerous small, round nuclei without definite cytoplasm. They were rather heavily stippled with chromatin. There was a definite connective tissue framework and a few small blood vessels.

DISCUSSION

Dr. George W. Bartelmez of our Department of Anatomy very kindly examined the sections of this tumor. He was of the opinion that the ciliated columnar epithelium which lined the cystic cavity was most comparable to the epithelium of the respiratory system. The glands which were definitely mucous in character were comparable to the tracheal glands. Admittedly other possibilities must be considered. It would seem obvious that any relation between this tissue and ependyma can be ruled out definitely for several reasons: the epithelium was definitely a mucus-secreting structure as it contained numerous, large goblet cells, and the cells and lumen

Microscopic Findings

Fluid: The mucus-like fluid was smeared immediately after removal, fixed with alcohol and stained with hematoxylin and eosin (Fig. 3). It contained numerous cells which varied greatly in size from small cells with very dark staining, round nuclei, and a small amount of granular, eosinophilic cytoplasm, to enormous cells with nuclei as large as the entire area of the smaller cells. The nuclei contained a heavy chromatin network. The cytoplasm of the large cells was granular, slightly eosinophilic and often markedly vacuolated so as to give the appearance of foam cells. The larger cells were often multinucleated, and contained from two to four nuclei. They also tended to group together in rows of two to five cells. Dr. William Bloom of our Department of Anatomy examined this smear very carefully, and was of the opinion that the larger cells were macrophages. He also thought that all stages of transition from lymphocytes to macrophages were demonstrable. An occasional polymorphonuclear leukocyte was also present. There were no ciliated cells in the fluid, as was noted in Kubie and Fulton's case.²

Cyst Wall: The cyst was lined with a columnar epithelium a single cell in thickness in most places; however, at points, it assumed a pseudostratified appearance (Fig. 7). The cells were moderately tall columnar in type with basal, oval-shaped nuclei. There was a definite basement membrane. The external surface of the cells was covered with numerous long cilia (Fig. 5). There was no evidence of the brush border which is typical of intestinal epithelium. Interspersed among these columnar cells were many typical, large, swollen goblet cells (Fig. 2). In sections stained with mucicarmine the contents of the goblet cells and the material found in the lumen of the cyst stained bright red. Underlying the epithelium and occupying the core of the papillae there was a very vascular, loose connective tissue. In this connective tissue layer were to be found many glandular structures. These structures were composed of a collection of alveoli lined with a single layer of columnar cells and many goblet cells. These glands frequently lay beyond this loose connective tissue in the underlying layer which was a thick band of smooth muscle, or even in the layer of connective tissue which blended with the overlying adipose tissue, or with the remainder of the tumor.

The remainder of the tumor, *i. e.*, that part ventral to the cyst, was composed of a rather loose connective tissue framework in

sanguineous fluid intermittently until the time of admission. There were no abnormal neurological findings. At operation an apple-sized, soft, elastic, sessile tumor was removed and the pedicle ligated as it left the sac. The underlying spinal canal defect was closed by osteoplastic resection. Recovery was practically uneventful. Histological examination proved the lesion contained atypical and undifferentiated muscle fibers, convoluted glands resembling those of the large intestine more than the small, mucous glands, as proved by staining methods, lymphatic tissue and nerve filaments.

It would seem very likely, therefore, that we were dealing in the case reported here with a trigeminal, congenital neoplasm — a teratoma — a “twin” which had not gone on to full development. Such a division of the ovum, so as to produce two organisms, might have occurred at any time during the presence of the primitive streak, *i. e.*, up to the fourth week following fertilization (Bartelmez⁶), and would have been due to the physiological isolation of the two halves of the primitive streak.

SUMMARY

1. A case of a dorsal lumbosacral teratoma in a Mexican female of 15 months is reported. Two additional cases have been collected from a review of the literature. All of the cases have been associated with spina bifida of the lower lumbar region. None of the three has had neurological abnormalities.

2. The tumor in the authors' case is composed of a mucus-containing cyst lined with ciliated, columnar epithelium thought to be comparable to respiratory epithelium, smooth muscle, connective tissue, bundles of myelinated nerve fibers, a Vater-pacinian corpuscle, a ganglion containing typical dorsal root or sympathetic ganglion cells, and lymphoid tissue.

3. The teratoma is thought to be an undeveloped “twin” due to division of the primitive streak during the first four weeks of embryonic life.

of the cyst contained material which stained well with mucicarmine; also, there was a clear-cut, limiting membrane to the epithelial layer; and further, the mucous glands in the deeper structures did not correspond with any structure related to ependyma. The possibility that this is comparable to intestinal epithelium must also be considered; however, the absence of the typical brush border is against that possibility. The epithelium, although not greatly different from that of the genital tract, contained numerous goblet cells which are seen in the cervix, but not found in the Fallopian tube or in the body of the uterus. The cells in the neoplasm, however, are not as tall as those seen in the cervix or in the glands of the cervix.

With the association of this mucous epithelium, the smooth muscle, connective tissue and lymphoid tissue, the myelinated nerve fibers, the Vater-pacinian corpuscle and the ganglion, we obviously are dealing with a trigeminal congenital neoplasm — a teratoma.

Such structures, as a diligent search through the literature since 1800 revealed, are exceedingly rare in the lumbosacral region. Teratomas in the region of the coccyx, anterior to the sacrum, even connected with the neural structures in the spinal canal through a defect in the anterior portion of the sacrum, are relatively common. It would appear that these develop from remaining vestiges of the neurenteric canal and postanal gut. However, no such structure is so situated as to explain the origin of this tumor, or of the obviously closely related teratomatous spinal cord cysts of Kubie and Fulton,² and the intradural teratomatous tumor of the spinal cord reported by Hosoi.³ The first reported case similar to the authors' is that of Sonntag⁴ in 1925. His patient was a male child 4 months' old, with a midline lumbosacral tumor. The illustration of his patient is almost identical with Fig. 2. The tumor at operation was found to hang on a stalk as thick as a child's finger, which disappeared through a fascia-muscle-bone defect to attach to the dura. Telangiectasia, fibrous tissue, fatty tissue and cartilage were described in the tumor, but detailed histological study was not reported.

The second case was Aloï's⁵ patient — a female, aged 19 years, who had had a lumbosacral tumor associated with spina bifida since birth. It had been considered inoperable at birth, and in about a year the tumor reached the size of a pigeon's egg. It ruptured spontaneously and drained clear, watery fluid. It then drained sero-

DESCRIPTION OF PLATES

PLATE 60

- FIG. 1. 15 months' old Mexican child with a teratoma in the lumbosacral region.
- FIG. 2. Portion of cyst wall containing many goblet cells. Hematoxylin and eosin. $\times 200$.
- FIG. 3. Smear of the fluid from the cyst showing cells of various sizes including one large vacuolated cell (foam cell) in the upper right-hand corner. Hematoxylin and eosin. $\times 200$.
- FIG. 4. An atypical Vater-pacinian corpuscle. In the lower portion is a group of concentric rings enclosing a clear space, the inner bulb. Hematoxylin and eosin. $\times 200$.
- FIG. 5. Section of the cyst wall showing mass of cilia. The epithelium at this point is pseudostratified. Hematoxylin and eosin. $\times 1200$.

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PLATE 61

FIG. 6. Ganglion containing numerous ganglion cells, each surrounded by several capsular cells. The two bundles of myelinated nerve fibers are present in the lower part of the illustration. Hematoxylin and eosin. $\times 100$.

FIG. 7. Portion of cyst wall lined by simple columnar and pseudostratified columnar epithelium. There is a group of goblet cells in the upper left-hand corner. Hematoxylin and eosin. $\times 150$.



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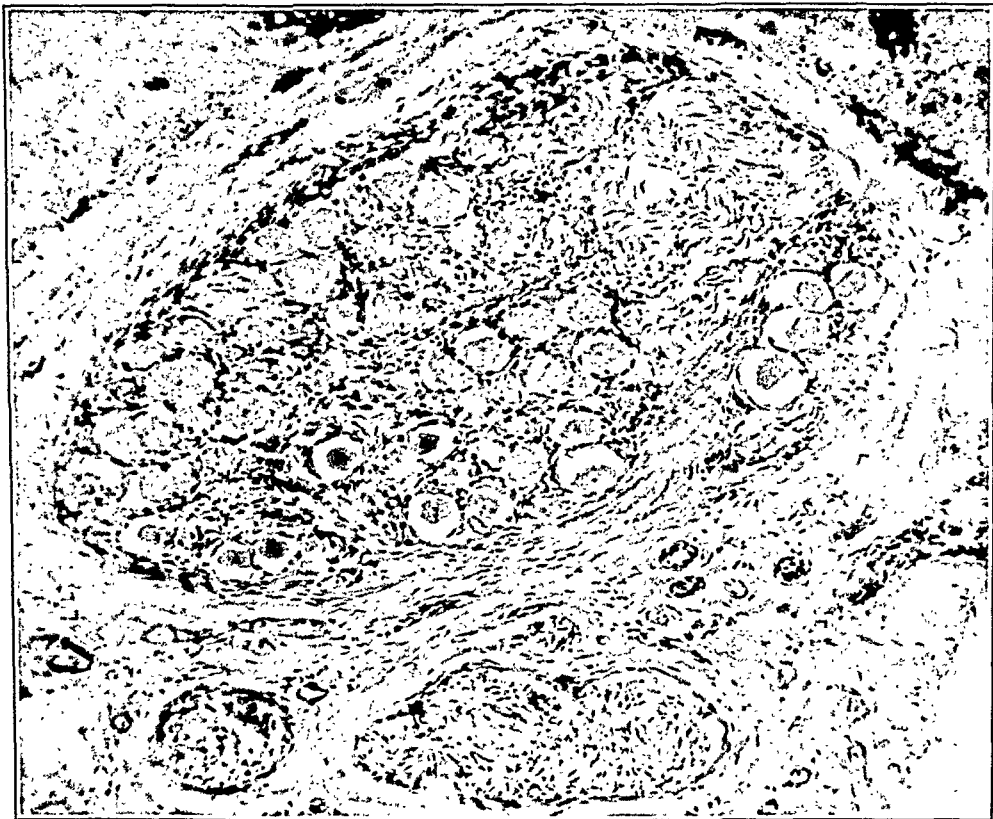
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He also continued to take digitalis in maintenance doses, and remained comfortable until May 11, 1930.

On that day, following a meal, he experienced cramp-like pain in the lower abdomen, soon followed by retching and vomiting. He reported to the cardiac clinic on May 16. At the clinic his temperature was noted as 102.5° F and he appeared acutely ill. He went home to bed, noticed marked weakness, gastric distress, eructation and distention. During the week prior to admission, he noted the sudden appearance of a painful red spot on the dorsum of his left foot, which persisted until the day of admission. Following this, he experienced sharp, sticking pain in the large toe of the same foot. This lasted twenty-four hours. The next day he felt a similar pain in the large toe of the right foot. Four days before admission he noted a painful red spot in the palm of the right hand. The day before entrance he felt extremely short of breath and complained of precordial pain and orthopnea.

In his past history the only illness he knew of, besides the rheumatic affections noted above, was pneumonia with empyema in early childhood.

His family history was negative.

Physical Examination: On admission the patient appeared well developed, fairly well nourished, but acutely and chronically ill. He exhibited dyspnea at rest and orthopnea of choice. The head jerked with each systole of the heart. Pallor was marked, but there was no notable tinting of the skin. The conjunctivae were the seat of numerous petechial hemorrhages with white centers. The sclerae were bluish. The pupils were round, regular, and reacted to light and accommodation. In the fundi of both eyes numerous retinal petechiae were seen. The nose was negative for abnormalities. The teeth were in fair condition and the tongue was moist. Few petechiae were visible in the mucous membrane of the hard palate. The veins of the neck were not distended. Markedly accentuated carotid pulsations were visible.

The chest was deformed on the left side due to retraction of the third and fourth ribs and interspaces in the anterior axillary line. There was dullness over the right base, and a leathery rub could be heard on the left side from the angle of the scapula down. It had no relation to respiration and was thought to be pleuropericardial. Many moist râles were audible over the base of the left lung.

The point of maximum intensity of the cardiac impulse was difficult to locate. The impulse was diffuse and was visible in the sixth interspace in the midaxilla, 13.5 cm. to the left of the midsternum. There was a visible systolic retraction in the third and sixth interspaces. A systolic thrill was palpable over the pulmonary area, none over the apex. Slight tenderness was elicited over the precordium. The first sound at the apex was replaced by a murmur which began in mid-diastole and lasted through systole. It was musical in quality. P₂ was greater than A₂ and accentuated. Over the base of the heart a roughened systolic murmur was heard and an early blowing diastolic murmur which was loudest at Erb's point. The ventricular rate was 120 and of regular rhythm. The pulse was of the Corrigan type, rate 120. A pistol-shot sound was heard over the brachials and femorals. The blood pressure was 160/0.

There was moderate distention and tympanites of the abdomen. The liver and spleen were not felt, and there were no signs of fluid. No edema of the extremities, or clubbing of the fingers or toes, was noted. There was an elevated, tender, red node, 1 cm. in diameter, in the center of the palm of the right hand. A similar node which was not tender was seen on the dorsum of the left foot. There were many petechiae in the skin of both the upper and lower extremities.

MICROCOCCUS PHARYNGIS SICCUS ENDOCARDITIS *

IRVING GRAEF, M.D., CLARENCE E. DE LA CHAPELLE, M.D., AND
MARGARET C. VANCE

(From the Third (N. Y. U.) Medical Division and the Third Division Pathological Service, Department of Pathology, Bellevue Hospital, New York, N. Y.)

The following case of acute vegetative endocarditis is reported in order to call attention to an instance of a fatal bacterial infection in man caused by a microorganism ordinarily considered non-pathogenic for man, namely, *Micrococcus pharyngis siccus*. It was first identified by Von Lingelsheim. He found it to be a common inhabitant of upper respiratory passages of normal individuals. It was included by Bergey¹ in the Gram-negative cocci of the genus *Neisseria* and named the *Neisseria sicca*.

Schultz² in 1919 recorded the first case that we have been able to find of bacterial infection in man ascribed to this microorganism. His was a case of acute vegetative endocarditis with multiple secondary foci of involvement in a previously healthy young adult man. In his case the microorganisms were recovered from the blood stream in pure culture twice during life, and from the vegetations and the spleen at autopsy. It is to be noted that the heart at post-mortem examination showed no evidence of preëxisting disease.

CASE REPORT

Clinical History: A. C., a doorman by occupation, 27 years of age, unmarried, born in this country, entered Bellevue Hospital, May 25, 1930, for the third time in five years. He had been a regular attendant of the Adult Cardiac Clinic of Bellevue Hospital since 1925, when his symptoms of heart disease became manifest.

At the age of 17 he had a mild attack of rheumatic fever associated with pneumonia and pleurisy. A severe attack of rheumatic fever lasting five months occurred at the age of 22. Between the first and second attacks he had had frequent joint pains. In 1925, following the second attack, he suffered his first heart failure and was admitted to the hospital with signs and symptoms of carditis and congestive heart failure. He recovered after a month and then attended the cardiac clinic. There he was given maintenance doses of digitalis which he continued to take thereafter. He was then able to do light work until July, 1929. At that time he had a recurrence of carditis and congestive heart failure. After seven weeks he was discharged from the hospital, improved. He continued to attend the cardiac clinic and to work as a doorman at a theater.

* Received for publication February 11, 1932.

The lungs were edematous and hyperemic. The visceral pleurae showed numerous petechial hemorrhages. There were no other changes.

The heart was markedly enlarged and weighed 945 gm. All the chambers were dilated. On section the myocardium appeared to be infiltrated by a moderate number of diffusely scattered, small, white and yellowish white deposits of pin-head size. The myocardium of both ventricles was considerably hypertrophied. The tricuspid valve and its chordae tendineae appeared normal. The pulmonary valve was slightly thickened and showed beginning fusion of the cusps. Small, firm, verrucous vegetations were visible between the anterior and posterior cusps. The mural endocardium in the left auricle above the mitral valve showed several small verrucous vegetations with definite thickening of the entire endocardium. The mitral valve (Fig. 1) was diffusely thickened, rigid, moderately stenosed, and measured 11.5 cm. at the base. Its chordae tendineae were somewhat thickened and shortened. A large, friable, reddish brown vegetation measuring 2.5 cm. by 7 cm. by 3.5 cm. was found attached to the auricular surface of the aortic cusp of the mitral valve. The aortic valve showed thickening and rolling of the edges with fusion of the commissural edges. In addition there was a row of fine verrucous vegetations on the ventricular surface. Beneath the valve the endocardium was markedly sclerosed. Several small creeping vegetations were found on the chordae tendineae of the mitral valve. The aorta and coronary vessels appeared normal.

The spleen appeared markedly enlarged, weighing 690 gm. It was soft and very friable on section. A small, firm infarct was found along the inferior border.

The liver was considerably enlarged, weighing 2600 gm. Its capsule was smooth and intact. On section the markings of chronic passive congestion were visible. The gall-bladder appeared normal.

The gastro-intestinal tract was normal except for the presence of numerous petechial hemorrhages on the serous surface of the intestine.

The kidneys were of normal size and their capsules stripped easily, leaving a smooth surface which showed many small petechial hemorrhages. A small, anemic infarct was found at the superior pole of the left kidney. On cut-section numerous pin-point and linear streaked hemorrhages were seen.

Laboratory Data: Urinalysis showed a specific gravity of 1020, albumin +, no sugar, no acetone, many white and epithelial cells, numerous red blood cells, and many hyaline and granular casts.

The urine sediment count (Addis) for a 12 hour specimen gave the following results: pH 5.0, specific gravity 1020, albumin +, red blood cells 1,799,900, white and epithelial cells 4,240,500, and casts 3,198,000 (chiefly granular).^{*} The blood Wassermann was negative. The non-protein nitrogen of the blood was 35 mg. per cent, sugar 90 mg. per cent. The hemoglobin was 70 per cent (Dare), the red blood cells numbered 3,828,000, the leucocyte differential count was polymorphonuclear leucocytes 87, lymphocytes 10, and monocytes 3.

Blood cultures, five days and two days ante mortem, yielded a Gram-negative coccus in pure culture.

Electrocardiographic examination five days before death showed sinus tachycardia with arrhythmia and incomplete intraventricular block (partial bundle branch block). (Electrocardiograms taken a year previously showed only changes in the R-T segments and occasional tachycardia.)

Course: On admission the temperature was 104° F, the pulse rate was 110. Subsequently the temperature ranged between 102° and 105° for six days. The pulse range was between 100 and 130. His toxic state grew more profound. Petechiae appeared in showers in the skin of the shoulder, arms, chest and neck. He became stuporous and died on May 31, 1930, six days after admission, with terminal pulmonary edema.

Clinical Diagnosis: Cardiac † (A) *Etiological:* Rheumatic fever, inactive, active? Gram-negative coccus.

(B) *Anatomical:* Enlarged heart, adherent pericardium, mitral stenosis, mitral insufficiency, aortic insufficiency, bacterial endocarditis.

(C) *Physiological:* Sinus tachycardia.

(D) *Functional:* Class III.

AUTOPSY FINDINGS

There was moderate edema of the lower extremities. Numerous petechial hemorrhages were seen in the conjunctival sacs, buccal mucous membranes and in the skin of the entire body. The peritoneal cavity contained a slight increase in fluid, which was clear and straw-colored. Both pleural cavities contained a slight amount of serosanguineous fluid. The pericardial sac was entirely obliterated by old fibrous adhesions. Where it could be stripped the revealed surface contained numerous petechial hemorrhages.

^{*} Normal values for 12 hour specimens should not exceed: red blood cells, 500,000, white blood cells, 1,000,000, and casts up to 5000.

† Cardiac diagnosis conforms to the nomenclature recommended by the American Heart Association.

Sections of the aorta (Fig. 6) were interesting because of the finding of flame-shaped scars extending from the adventitia into the media, interrupting the elastic lamella. These were considered characteristic of the healed stage of rheumatic aortitis as described by Pappenheimer and Von Glahn.³ Many of the nutrient arteries showed sclerotic and endarteritic changes.

Sections of the other organs confirmed the gross diagnosis.

Final Pathological Diagnoses: Acute bacterial endocarditis of the mitral valve and its chordae tendineae; chronic valvulitis of the mitral, aortic and pulmonary valves; acute verrucous endocarditis of the mitral, aortic and pulmonary valves, and of the left auricle; healed aortitis (rheumatic); hypertrophy and dilatation of the heart; multiple abscesses of the myocardium; fibrosis of the myocardium; aortic insufficiency, mitral insufficiency; adhesive pericarditis; chronic passive hyperemia of the lungs; pulp hyperplasia of the spleen, infarct of the spleen; acute focal embolic nephritis, anemic infarct of the left kidney; chronic passive hyperemia of liver; petechial hemorrhages of the skin and serous membranes; edema of the feet.

BACTERIOLOGICAL FINDINGS

Blood culture done five days before death yielded a pure growth of a Gram-negative coccus in broth and on blood agar plates. An identical strain was recovered from the heart's blood at autopsy, and from the fresh vegetation taken from the mitral valve. As noted above in the microscopic findings, the sections of the vegetation showed the colonies in it to be Gram-negative. On blood agar plates the colonies were smaller than meningococcus colonies. They were firm, tenaciously adherent to the medium; in broth and salt solution they sedimented spontaneously. After 48 hours the surface of the colonies became corrugated.

The colonies attained the size of 2.5 to 3 mm. They were slightly irregular in outline but had smooth borders. They were somewhat raised, glistening, opaque and colorless. After prolonged growth they could be removed as dried masses.

The organisms grew on the surface of all media, exhibiting constant characteristics. No pigment was formed at 37.5° C, at room temperature, on potato, Loeffler's serum or plain agar media. Culture in daylight and darkness was not accompanied by pigment production. Gelatin was not liquefied.

Examination of the other organs showed no notable pathological changes.

Bacterial cultures were made of blood of the inferior vena cava and fragments of the vegetation on the mitral valve.

MICROSCOPIC FINDINGS

Heart and Great Vessels: Study of the mitral valves (Figs. 2, 3 and 4) revealed a densely sclerosed valve with moderate cellular infiltration. These cells were chiefly lymphocytes. In places there was evidence of a fresh inflammatory process with areas of edema and surrounding collections of polymorphonuclear leucocytes, histiocytes and a few lymphocytes. In some sections verrucae were seen which were indistinguishable from those seen in rheumatic verrucous endocarditis. On the auricular endocardium, some distance from the vegetation, were found small verrucae composed of fibrin on a proliferated base containing many histiocytes.

Sections of the mitral valve, which included the vegetation, showed the base to be fairly well organized by the deposition of many fibroblasts, which could be seen growing into the thrombotic vegetation. The vegetation itself was composed of deeply staining masses of fibrin, leucocytes and large clumps and colonies of bacteria, which were found to be *Gram-negative when stained by the MacCallum-Goodpasture method*. (A control section was stained by the same method.)

Sections of the aortic and pulmonary valves confirmed the gross diagnosis of chronic valvulitis and acute verrucous endocarditis.

Sections of the myocardium revealed extensive changes throughout. Dense, acellular, fibrous connective tissue was found around the blood vessels. Interstitial connective tissue was increased throughout. In areas, irregular connective tissue scars were seen interrupting muscle bundles and replacing them. The intact muscle was composed of hypertrophied fibers and hypertrophied nuclei. Numerous large and small miliary abscesses were found in the myocardium of both ventricles and the left auricle (Fig. 5). No Aschoff bodies were seen. Bacterial stains of sections through the abscesses were unsatisfactory because of the large amount of pyknotic nuclear material. Occasional areas of necrosis were found, which showed invasion by lymphocytes and proliferation of fibroblasts. Several small branches of the coronary arteries showed purulent thrombi.

SUMMARY

A case of bacterial endocarditis (malignant) caused by the *Micrococcus pharyngis siccus* is presented in a human subject with pre-existing valvular disease of rheumatic origin.

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DESCRIPTION OF PLATE

PLATE 62

- FIG. 1. Close-up photograph of the mitral valve. Note the verrucae along the margin of the anterior leaflet; also note the mural lesion in the auricle above the large vegetation.
- FIG. 2. Low power photomicrograph of a section through the mitral valve and vegetation. $\times 10$.
- FIG. 3. Low power photomicrograph of a section through the anterior cusp of mitral valve showing verruca composed of fibrin deposited on base consisting of proliferated histiocytes, lymphocytes and fibroblasts in a hyalinized and sclerosed valve cusp. $\times 75$.
- FIG. 4. Photomicrograph to show clumps and colonies of cocci in the vegetation on the mitral valve. $\times 600$.
- FIG. 5. Low power photomicrograph of a section of the left ventricle showing abscess formation in the myocardium. $\times 75$.
- FIG. 6. Low power photomicrograph of a section of the aorta showing scars in the outer layers of the media, with interruption of the elastica, and slight round cell infiltration. Van Gieson-Weigert elastica-iron hematoxylin. $\times 75$.

In peptone water indol was formed. The organisms grew slowly in 2 per cent dextrose agar under anerobic conditions and formed no gas.

The following table indicates the behavior with sugars, inulin and milk:

Filtered maltose	Acid	Gas
Maltose	"	"
Dextrose	"	"
Levulose	"	"
Saccharose	"	"
Lactose	—	—
Dextrine	—	—
Mannite	—	—
Inulin.....	—	—
Milk	—	—

One rabbit was inoculated intravenously with 1.5 cc. of a 24 hour broth culture, one guinea pig was injected intraperitoneally with 1 cc., another guinea pig was given 2 cc. intraperitoneally with no ill effects, and one mouse was injected intraperitoneally with 0.5 cc. The animals were killed after two weeks and gross and microscopic examination of their tissues showed no pathological changes.

COMMENT

The portal of entry for the infecting organism in this case is obscure. We are forced to assume that a bacteremia occurred some time prior to the onset of symptoms and that the organisms became implanted on the deformed mitral valve. While it is generally held that pathogenic organisms frequently become implanted on diseased heart valves, it is unusual for non-pathogenic bacteria to do so, or even to become the etiological factors for vegetative endocarditis. However, instances have been recorded of such endocardial lesions associated with other non-pathogenic bacteria.

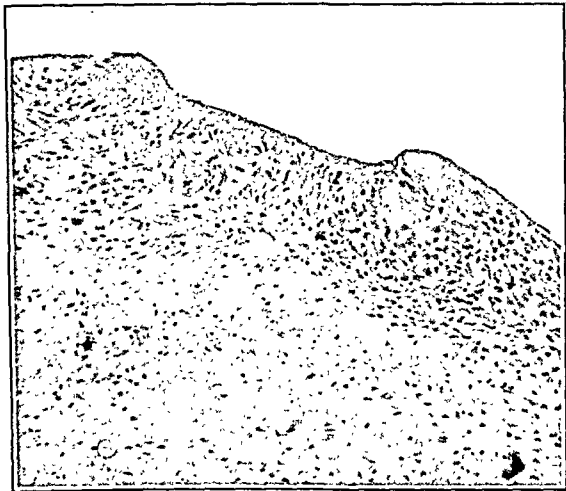
As noted above, Schultz's case was an infection apparently *sui generis*. Of interest in connection with this type of case is a report by Coulter ⁴ in 1915 of bacterial vegetative endocarditis due to an unknown Gram-negative micrococcus, and the case recently reported by Dickar ⁵ due to the *Bacillus acidi lactici*. In both cases reported no preëxisting disease of the heart was found. One can only speculate on the factors which seem to render these organisms pathogenic for man in special circumstances.



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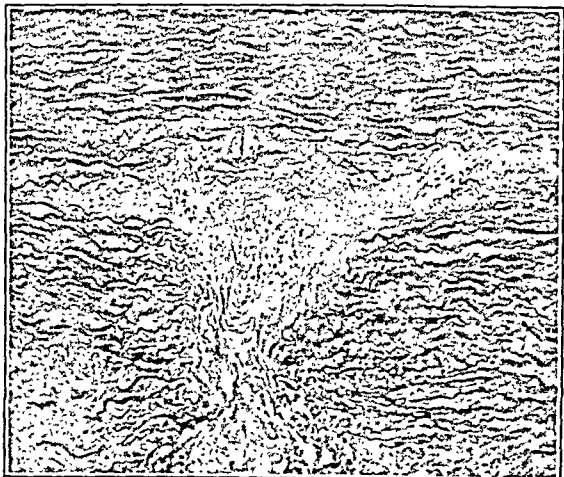
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the repair of small lesions in the liver these cells migrate toward the site of injury and contribute to the formation of a delimiting capsule surrounding the injured zone.⁴ Comparable reactions have been displayed by these cells of the liver in the presence of autogenous transplants such as kidney and muscle.⁵

Although the literature on the cytological effects of radium emanations is enormous, we are not aware of recorded studies on the reaction of the histocytes in the liver to such radiant energy. Accordingly this study was undertaken to determine the extent of injury within the immediate field of irradiation, as well as the degree of recovery and the restorative powers of local histocytes more remote from the source of this energy. Only healthy white rats of our colony, ranging in age from 4 to 6 months and in weight from 125 to 175 gm. were used.

EXPERIMENTAL METHOD

The gold seeds used to introduce the radon into the liver were about 3 mm. long and 0.6 mm. in diameter (outside). The wall of these tiny cylinders, about 0.2 mm. thick, absorbs all but about 0.36 per cent of the beta rays, but permits the passage of 82 per cent of the gamma irradiation.⁶ Using aseptic technique, the liver was delivered through a median-line incision and by means of a suitable spinal puncture needle the radon seed was introduced into the hepatic parenchyma to a level about 0.5 cm. below the capsule. Bleeding was slight and easily controlled. Each radon seed contained approximately 1.16 millicurie.

In a series of animals used as controls operation was performed in the same manner, and valueless or inactive gold seeds, but otherwise identical in every respect, were introduced into the corresponding lobe of the liver. The control and the irradiated animals were killed at intervals ranging from six hours to three months. Sections of the liver containing the gold seeds were excised, fixed, and stained with hematoxylin and eosin, Van Gieson's stain and Mallory's stain for connective tissue.

In order to follow the reaction of these cells to the inactive seeds as well as those containing radon, it was desirable to mark them in such a way as to facilitate identity. The extensive phagocytic activity of these cells and the retention, for long periods of time, of engulfed materials provided adequate means for their subsequent

EFFECT OF RADIUM EMANATION ON THE HISTOCYTE IN THE LIVER OF THE WHITE RAT *

GEORGE M. HIGGINS, PH.D., AND J. C. THOMAS ROGERS, M.D.

*(From the Division of Experimental Surgery and Pathology, The Mayo Foundation,
Rochester, Minn.)*

The histocytes of the liver lie along the hepatic trabeculae and in the portal spaces. They may be closely attached to the reticular network of the lobule or they may project well into the lumen of the sinusoid. Frequently, in either normal or pathological conditions of the liver they may become detached and migrate through the hepatic parenchyma to the portal spaces, or they may enter the blood stream and pass to other organs of the body. In a normal liver these histocytes are distributed rather irregularly along a sinusoid, but are slightly more abundant in the median and peripheral portions of the lobule. They are ordinarily elongated or spindle-shaped, but when stimulated to activity they assume various forms so that the stellate outline originally employed to describe them is often seen.

Many functions are attributed to these littoral cells. Chief among these, perhaps, is that of defense, for their phagocytic capacity for either organic or inorganic materials is truly enormous. The contribution of these histocytes to pigment metabolism, either by the intracellular digestion of erythrocytes or by engulfing dissolved hemoglobin, is well known. Data concerning the relation of these cells to normal and pathological lipin and carbohydrate metabolism are gradually becoming available.

We shall not attempt to review the extensive literature covering the various pathological conditions in which these littoral cells react, for it is sufficient for our purpose to mention only those related in a measure to this study. Mallory,¹ long ago, described the marked proliferation and the extensive phagocytosis of blood cells in typhoid fever. Similar conditions maintain in malaria, kala-azar, typhus fever, subacute bacterial endocarditis and chronic streptococcal infections.² In inflammatory processes these cells desquamate, transform into polyblasts and fibroblasts, and in chronic silica poisoning the formation of connective tissue has been ascribed to them.³ In

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portal spaces, for there was evidence to indicate first an accumulation of histocytes in adjacent portal spaces and thence a migration to the lesion. Graphite-laden cells within this wall varied in size and shape. Many of them were spherical or ovoid, whereas many others were attenuated and already exhibited the tendency to their subsequent development.

The effectiveness of the irradiation by the radon seed was clearly seen at the end of the third day,⁷ and the induced lesion was in marked contrast to the reaction in the liver of the control animals. The zone of injury was triple the depth of that observed at the end of the first day, and there was no differentiation into an inner and outer zone such as characterized the lesion which developed around the control seed. The injured area, equal in radius to the diameter of the seed, consisted of much fibrin, necrotic material, free graphite and many necrotic histocytes. The region of intense injury continued rather imperceptibly into the peripheral normal parenchyma where vacuolization of the cells and a pale staining reaction indicated injury to hepatic cells. Histocytes immediately peripheral to the region of maximal injury in many instances had been broken down and had given up their pigment granules as a result of the irradiation. In regions more remote from the active seed, beyond the immediate influence of the irradiation, graphite-laden histocytes showed signs of some activity, although much less than that encountered at the same interval in the control animals. In the irradiated livers histocytes did not accumulate in the portal spaces or migrate along paths to the source of the irradiation.

Recovery from the injury induced in the control animals, initiated by the third day, was essentially complete by the third or fourth week. Necrotic tissue incident to the insertion of the seed had been removed and scar tissue remained to indicate the site of the foreign body implant. The lobules in the parenchyma adjacent to the lesion were practically free of graphite-laden histocytes, and new mononuclear cells, probably of local as well as of extraneous origin, had taken their places along the sinusoids. Portal spaces, however, contained extensive accumulations of graphite-containing giant cells, fibroblasts and histocytes, and the scar tissue at the site of the seed implant contained much carbon pigment.

The destructive effect of the radon seed on the liver continued for at least five or six weeks, varying somewhat in the animals examined.

identification. Two intravenous injections of 0.5 cc. of a graphite preparation on successive days, originally described by Drinker and Churchill, was sufficient to distend these cells and make their subsequent identification in histological sections satisfactory (Fig. 1).

OBSERVATIONS

Trauma was, of course, incidental to the introduction of these seeds into the parenchyma of the liver, and slight hemorrhage, together with the formation of an area of necrosed hepatic parenchyma, was unavoidable in all animals. Accordingly the reactions sustained after twenty-four hours in both the control and the irradiated portions of the liver were essentially identical. A zone of compressed, necrosed hepatic cells, blood cells and fibrin surrounded the site of the seed, and scattered graphite from destroyed histocytes was distributed throughout. Any destructive effect of radon on the histocytes of the parenchyma, adjacent to the site of the seed, was certainly not apparent at the end of the first day, for in both the control and the irradiated parenchyma these cells were enlarged several times and projected well into the sinusoids. This apparent stimulation is probably due to the presence of foreign bodies in the form of gold seeds and to the engulfed graphite and the acacia in which the pigment was suspended.

After seventy-two hours, however, striking contrasts in the character and in the extent of the reactions of the parenchyma of the control and of the irradiated livers were observed. The injury induced within the liver containing the inactive seed showed marked recovery, and the degree of restoration at this time was comparable to that observed by Higgins and Murphy at seventy-two hours after the induction of small inflammatory reactions in the liver of rats. The lesion produced by the inactive gold seed was clearly divisible into two zones (Fig. 2). The inner of these consisted of the necrosed material incident to the insertion of the seed and was composed of fibrin, necrotic nuclei, polymorphonuclear leukocytes and fibroblasts. The outer zone, which effectively delimited the inner zone from normal peripheral parenchyma, was composed largely of graphite-laden mononuclear cells. These cells appeared to us to have migrated from their littoral position along the sinusoids to the lesion, and there contributed toward the formation of this cellular wall. Migration to the lesion took place along well defined paths in

not attempted to follow the reticulum of the hepatic lobule in this study, but it would be interesting to know whether these reticular fibers supporting hepatic parenchyma lose their identity when subjected to radium irradiation, as they do when exposed to the Roentgen-ray.⁷ Reticulum is probably a product of these local histocytes. It is common practice to designate Kupffer's cells as reticular cells, and the belief is current that reticulum bears some genetic relation to them. However, the exact origin of this fibrous network from reticular or littoral cells in the liver has never been established. Mallory and Parker⁸ derived reticulum of the liver lobule from fibroblasts of the stroma and not from these littoral lining cells. And yet there has been some evidence which would seem to indicate that these local histocytes may transform into fibroblasts. In cultures of adult mammalian connective tissue Maximow⁹ showed clearly that this silver-stained fibrillar network arises as the result of a precipitation or a transformation of some colloidal substance under the influence of unknown factors which originate within the cell. Undoubtedly reticulum in the hepatic lobule is precipitated from reticular cells in much the same way.

The reaction of the local histocytes to a foreign body, for in reality the control inactive gold seeds used in this experiment constituted a foreign body, was strikingly identical to that encountered in response to trauma induced in the liver by a small instrument. Those cells actually traumatized by the insertion of the seed were destroyed, and within twenty-four hours polymorphonuclear leukocytes appeared in abundance to remove the necrotic cells and the liberated graphite. During the time of this preliminary neutrophilic reaction local histocytes manifested activity, in that they retracted their processes, buckled into the sinusoids, and often were freed from their reticular attachments. The character of the stimuli inducing these reactions within regions considerably remote from the zone of injury is unknown. It is probably chemical. Many of these graphite-laden histocytes passed either directly to adjacent portal spaces or often directly to the lesion, and well defined paths leading to the necrotic zone were often clearly delineated by the heavily laden, graphite-containing mononuclear cells. Seventy-two hours after operation a rather well defined wall composed largely of these graphite cells had formed around the necrotic zone separating it

In general the maximal injury was induced some time between the fourth and the sixth week. The radius of the necrotic zone in these livers reached approximately twice the diameter of the gold seed by the fourteenth day and at least triple that extent by the thirty-fifth day (Fig. 3). At this time the transition from the necrotic zone to normal parenchyma was more abrupt than hitherto seen, and one may rightly conclude that the maximal effective destruction had been reached.

During these weeks there was but slight activity among the histocytes, either those closely adjacent to or even more remote from the lesion. There were no indications toward restoration such as occurred in the normal animals. In some of the rats studied at the fourteenth and the twenty-first day a slight migration of graphite-laden cells into adjacent portal spaces had occurred, but these were so infrequent as to merit slight recognition in the total processes of recovery.

The concluding observations, which were made at ten and twelve weeks after the introduction of the radon seeds into the liver, showed rather clearly that active histocytes were engaged in the process of recovery (Fig. 4). The hepatic parenchyma was free of graphite cells except in the portal spaces, and new littoral histocytes were distributed along the sinusoids in characteristic positions. The necrotic zone, although not completely absorbed, was reduced in extent and was completely isolated from the adjacent parenchyma by a wide wall or capsule formed of connective tissue fibers and mononuclear cells heavily studded with graphite. With connective tissue stains the fibers were clearly delineated and the distribution of graphite granules among them served to suggest that a transformation of the histocyte into connective tissue had taken place.

DISCUSSION

In this study of the reaction of the local histocytes in the liver to the radon, as contained in gold seeds, we have not attempted to follow the detailed cytological changes that were induced. We wished to know: (1) whether the littoral histocytes in the liver were any more resistant to radon than the parenchyma cell; (2) when the maximal injury had occurred, and (3) the recovery and the contribution of these cells to the restoration of the injured part. We have

inactive control seed. In fact, there was no initiation of restorative activity by peripheral histocytes until after the fifth week, when the maximal injury induced by the irradiation had been effected. The necrotic zone increased progressively in extent of its radius until at thirty-five days it was triple the diameter of the seed. At this time the fragmented histocytes were restricted largely to the peripheral portion of the lesion, whereas the medial and central portions consisted practically of fibrin and scattered carbon granules, together with a few mononuclear cells of unknown origin. Polymorphonuclear leukocytes were not present.

Recovery and organization of the lesion, as far as the participation of the histocytes was concerned, commenced some time between the fifth and the sixth week. Up to this time histocytes peripheral to the influence of irradiation had remained essentially inactive. Some retraction had occurred, but the desquamation, so to speak, or the migration so characteristic of these cells surrounding the control inactive seed during the first few days had not taken place. The duration of the destructive influences of the irradiation, if one is to judge by the cellular reaction in these livers, was approximately five to six weeks and is therein comparable to clinical data on the effectiveness of radon. Whether the pathological changes encountered were wholly due to the radon or to subsequent products of decay within these gold seeds is, of course, unknown.

Although the maximal injury was attained at five to six weeks, recovery and organization of the lesion were greatly retarded. There appeared to be prolonged effects, so that cells which normally would have acted quickly to stimuli responded but feebly. Observations made at seven, eight and nine weeks after the onset of irradiation showed only a slightly active histocytic system in the parenchyma surrounding the lesion. Gradually, however, a reaction ensued wherein these littoral cells, still containing the engulfed graphite, proceeded toward the lesion and formed a heavy wall around the now presumably inactive gold seed. In the last of the series of irradiated animals killed at twelve weeks after the insertion of the radon seed (Fig. 4), a reaction was noted, comparable in many respects to that seen in the livers of control animals after three days to one week. Here were large numbers of mononuclear cells laden with graphite, which together with connective tissue fibers formed a

from the peripheral normal parenchyma (Fig. 2). This reaction is identical to that seen by Higgins and Murphy in livers of rats seventy-two hours after the induction of slight trauma, and it confirms the observations of Biebl, who studied the reactions of the Kupffer cell to foreign bodies in the form of autogenous transplants such as kidney and muscle. Animals of the control series, which were killed at one week after placing the inactive seed into the liver, presented conditions in which the mononuclear wall was more compact. The peripheral parenchyma was relatively free of graphite cells and a more definitely compact wall separated the necrotic zone from the normal tissue. With acid fuchsin stains the connective tissue in the wall was delineated and graphite granules were abundantly distributed among its fibers. Many of the graphite-laden cells had retained a spherical or slightly ovoid contour, whereas many others were attenuated and resembled typical connective tissue cells. Here, then, were additional data which confirmed earlier opinions that hemophages of the liver may transform into or give rise to connective tissue cells. Following ligation of the major blood vessels to the kidney of the rat, Jordan, Kindred and Paine¹⁰ followed the activity of both fibroblasts and macrophages in the infarcted kidney. These cells had their own particular functions and there was no evidence that macrophages become transformed into fibroblasts, which, on the basis of our knowledge of active macrophages and fibroblasts, one should probably anticipate. It may be, however, that in later stages, as in advanced inflammation, such a transformation of macrophages into fibroblasts might take place. Cultures of organs have shown that large ameboid macrophages proliferate mitotically and finally transform into fibroblasts with an elaboration of the silver-stained, collagenous fibers. Reticular cells of the liver or of the spleen, however, may hardly be regarded as typical macrophages, although they are functionally phagocytic and may become macrophages when stimulated to activity by any foreign material.

When portions of the liver were irradiated by radon, histocytes were as susceptible to injury as any parenchyma or cell of the biliary duct. Within the necrotic zone seventy-two hours after operation, disintegrated or fragmented histocytes and scattered graphite mingled with the necrosed parenchyma and fibrin. There was no attempt at recovery, or organization, such as was seen around the

SUMMARY

1. A study has been made of the reaction of the histocytes in the liver to radon, as contained in gold radon seeds. Similar but inactive gold seeds were used in a series of control animals to determine the local reaction to such foreign bodies.

2. All local histocytes within the vicinity of the inactive gold seed responded immediately to the foreign body by retracting their processes, desquamating either into the sinusoids or migrating into adjacent parenchyma. Their function as littoral histocytes had apparently ceased and they were now concerned in an attack on the foreign body. Seventy-two hours after insertion of the inactive seed into the parenchyma an effective barrier had been formed around it by these actively migrating histocytes. Subsequently these cells contributed toward the formation of connective tissue.

3. All histocytes in the vicinity of the active radon seed were as susceptible to the emanation as the cells of the parenchyma. The extent of the destruction by the radon increased until the maximal injury was reached about the thirty-fifth day. Histocytes within the zone of injury were destroyed, and those immediately beyond did not manifest signs of restorative activity during this period. After the fifth week, however, a retarded restorative activity was manifested by the histocytes just peripheral to the zone of maximal injury. By the tenth or twelfth week these cells considerably remote from the radon seed, and which either had not been affected or only slightly affected by the radon, migrated rapidly toward the seed and contributed toward the formation of a wall comparable to that seen three days after the implantation of the control seed.

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compact wall separating the now inactive seed from the adjacent portions of parenchyma.

Accordingly, at twelve weeks after inserting radon into the hepatic lobes of rats cytological reactions were derived, rather closely resembling those seen at the end of one week in animals which had received the control seed. The maximal injury to the liver by the radon was apparently reached by the fifth or sixth week, and yet outlying histocytes made little if any contribution to recovery until the ninth or tenth week. Factors in this delay are not at once apparent, but we feel that they may be correlated with toxic influences set up by the destructive effects of the radon. Subsequently all histocytes not within the range of the effect of radon pursued a normal trend, in that they desquamated and migrated toward the foreign body and contributed toward the formation of the delimiting wall.

The evidence from this study sustains the opinion that reticular cells of the liver may transform into connective tissue cells. Normally such changes probably do not occur, and we should not suppose that Kupffer cells, while functioning in their normal capacity along the sinusoid, would produce connective tissue. Their function as Kupffer cells in regions more remote from the site of the radon was probably unimpaired during the interval the irradiation was effective, and there were no indications that fibroblasts had been produced along the sinusoids. After, however, the macrophagic function of these cells has passed and their function as a Kupffer cell in its relation to the physiological function of the liver has ceased, then these cells appear to acquire connective tissue potency and may transform into the highly specialized or differentiated fibroblast. Goldzieher and Hornick,¹¹ in a consideration of the source and the fate of the hyperplastic reticular cells in reticulosis, concluded that especially in the spleen, but also in the liver, definite fibrosis occurred. There was no proliferation of fibroblasts and the conclusion was reached that, under such conditions at least, Kupffer cells may transform into fibroblasts. These conclusions sustain our observations that when reticular cells of the liver no longer function as normal littoral cells, then transformations into the more highly differentiated fibroblast may take place.

DESCRIPTION OF PLATES

PLATE 63

FIG. 1. Liver of rat, following injection of graphite; littoral histocytes prior to insertion of radon seed are shown.

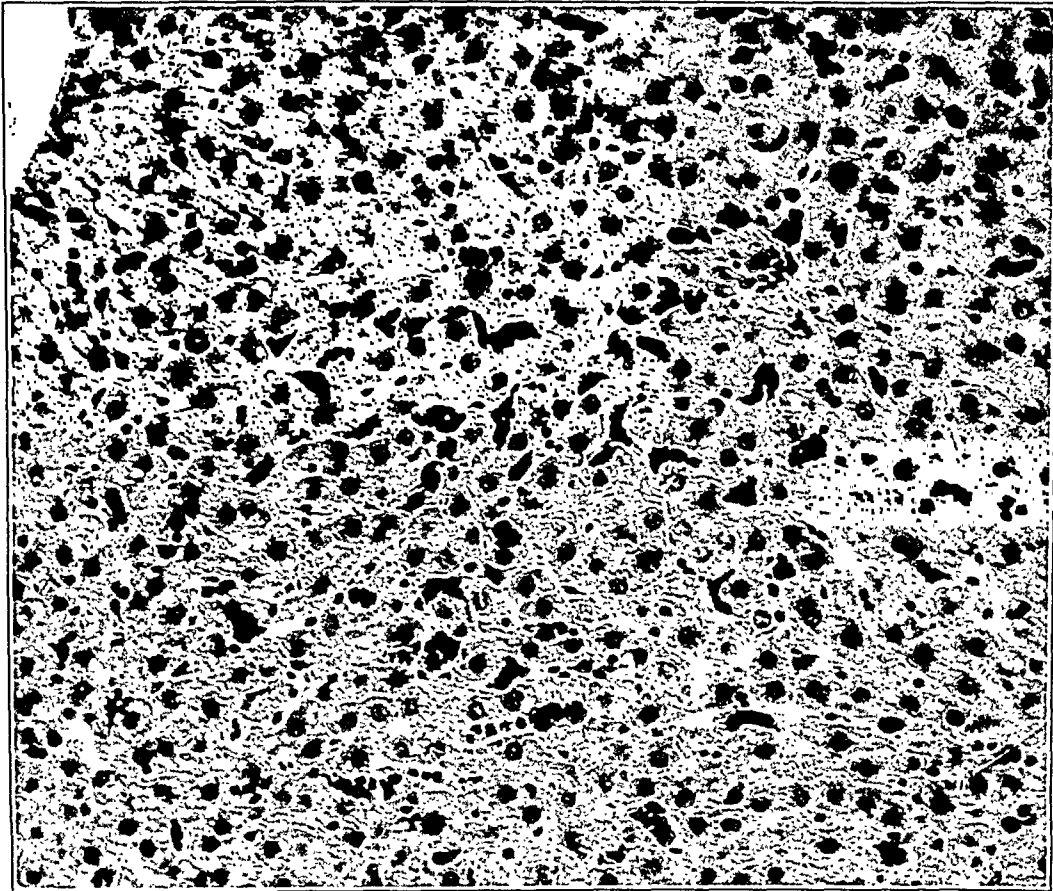
FIG. 2. Lesions in liver of rat seventy-two hours after insertion of inactive gold seed into hepatic parenchyma. Graphite-laden cells have migrated toward the lesion.

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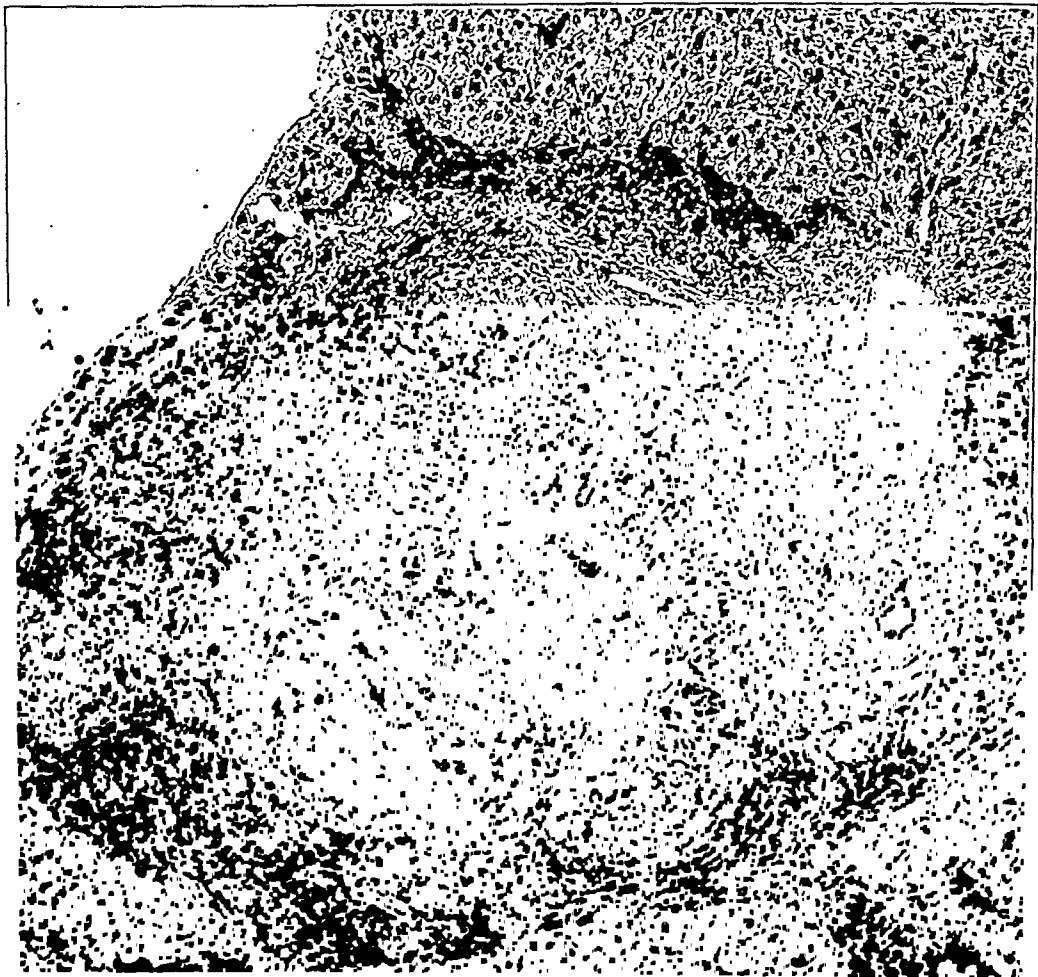
PLATE 64

FIG. 3. Lesion in liver of rat fourteen days after insertion of active radon seed into hepatic parenchyma.

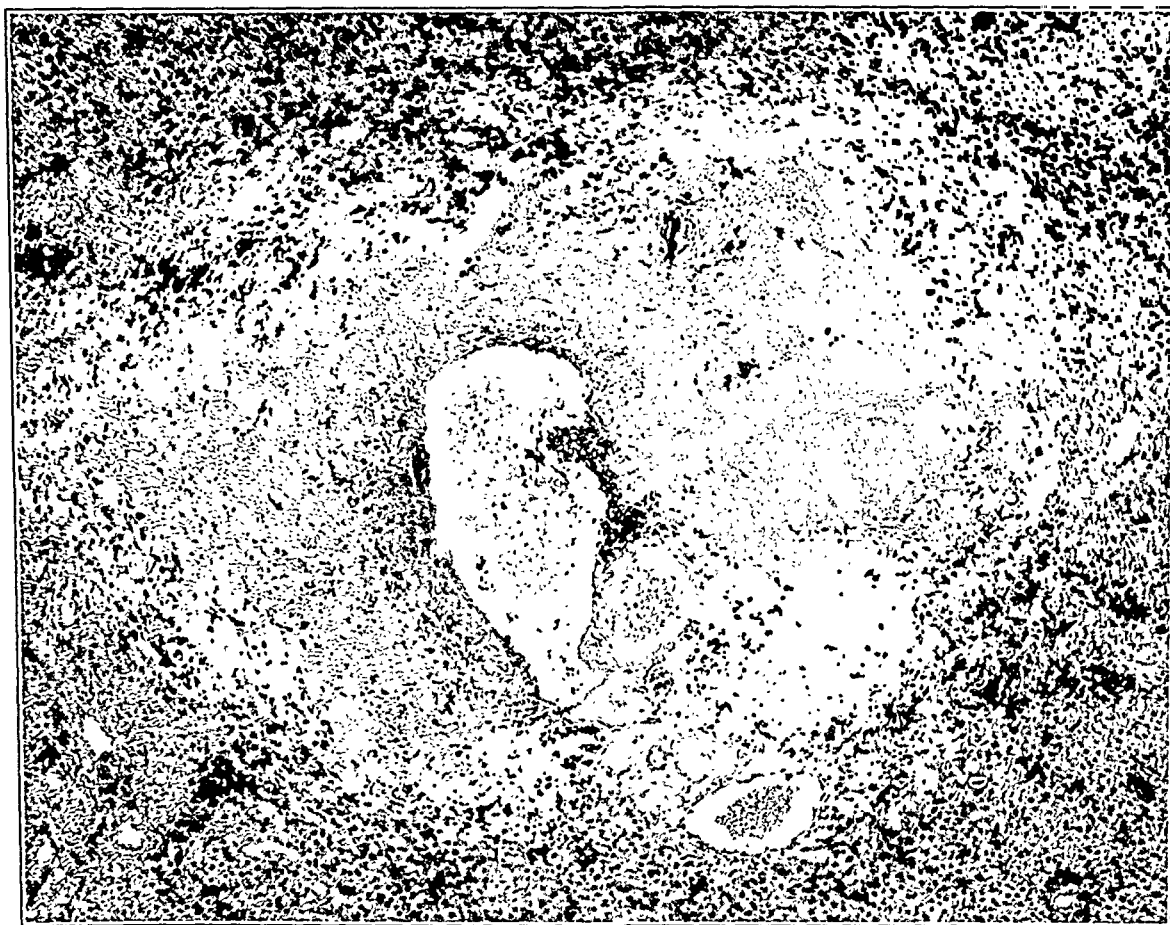
FIG. 4. Lesion in liver of rat twelve weeks after insertion of active radon seed into hepatic parenchyma.



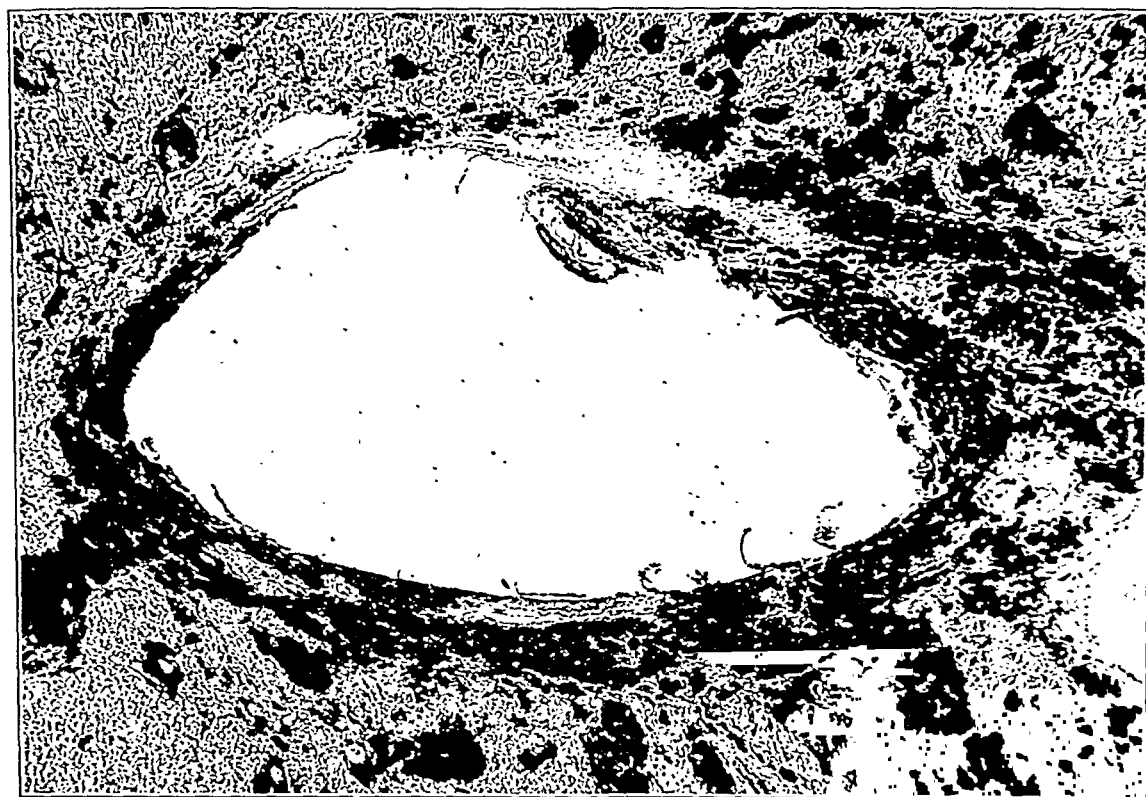
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field's perineurial fibroblastoma) and they cannot arise from Schwann cells, the neurectodermal nature of which is no longer open to discussion.

The following observations favor the second opinion. In certain examples of generalized neurofibromatosis, studying nerves of apparently normal dimensions, several authors (Verocay, Pick and Bielschowsky) have seen microscopic tumors composed of Schwann cells in proliferation. In the beginning this schwannian proliferation is accompanied by budding of collateral neurites. This budding of collaterals soon ceases but the multiplication of Schwann cells continues, accompanied by connective tissue proliferation. The specific cells of these tumors are connected in a syncytium like Schwann cells; they possess elongated nuclei of the same structure as schwannian nuclei, and they are grouped in networks, in bundles and in palisades. These latter structures are pathognomonic; they have only superficial resemblance to the groupings found in certain myomas and fibroblastomas. Between the cells there are fibers of peculiar nature and staining, coloring orange or pale pink with Van Gieson's stain.

Up to this point there is almost complete unanimity. The differences of opinion begin when assessing the importance of connective tissue participation in the formation of these tumors. While some hold it to be minimal, others believe it to be dominant or rather progressively dominant, increasing in proportion as the neurinoma grows larger. Following Verocay, Pick and Bielschowsky state that the growth of the endoneurial and perineurial connective tissue tends to disperse and stifle the Schwann cells and that they become as difficult to distinguish as the cancer cells of certain scirrhus tumors of the breast or of the stomach (!). According to Pick and Bielschowsky, the place of these tumors in the classification can be determined with certainty only by studying the initial changes.

The heart of the argument advanced by the first group is the presence in these tumors of collagen fibers of endoneurial type. They assume the connective tissue nature of the cells which, having produced the fibers, continue to live among them. For the second group these fibers create a difficulty. They extricate themselves by assigning the fibers to the connective tissue stroma of the schwannoma, a stroma of endoneurial origin, which to a greater or less extent may replace the neurectodermal cells and mask them if not

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EXPERIMENTAL AND SPONTANEOUS SCHWANNOMAS (PERIPHERAL GLIOMAS)*

P. MASSON

(From the Laboratory of Pathological Anatomy, Université de Montréal, Montreal, Canada)

INTRODUCTION

The following pages are addressed to that minority of pathologists who still refuse to admit that the fundamental constituent of the encapsulated tumors of the peripheral nerves is the schwannian syncytium, a neurectodermal structure, and not the mesodermal fibroblast. The great majority of pathologists who follow Bard, Durante, Francini, Verocay, Antoni and many others in accepting these tumors as schwannomas will not object to my reopening the discussion by offering new evidence to confirm their views. Let us first summarize the arguments advanced by the partisans of the connective tissue hypothesis and by those of the schwannian hypothesis as to the origin of these tumors.

The reasoning of the first group appears to be quite logical. The tumor cells are branched, anastomosing by their processes like connective tissue cells. They give rise to many fibrils which react like collagen or like reticulin and which are arranged like the fibers of the endoneurial framework. Furthermore one may distinguish delicate fibrils staining blue with Mallory's phosphotungstic acid hematoxylin, identical with the "fibroglia fibrils" of fibroblasts. These advocates assume that only fibroblasts, mesodermal cells, are capable of producing collagen and fibroglia fibrils. These tumors of the peripheral nerves should therefore be fibroblastomas (Pen-

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PART I

EXPERIMENTAL SCHWANNOMAS

THE SCHWANNIAN SYNCYTIIUM. THE SCHWANN CELL

Nowadays I believe everyone accepts the medullary origin of the Schwann cells, their departure from the neural crest and their migration along the primitive bundles of axones. In the adult medullated nerve fiber each internodal segment is enclosed in a delicate protoplasmic envelope, bounded externally by a cuticle — the membrane of Schwann. The protoplasmic envelope is applied closely to the myelin sheath and presents slight annular thickenings corresponding to the Schmidt-Lantermann incisures. In the middle of each internodal segment there is a slight thickening of the protoplasmic envelope. This thickening contains an oval nucleus with fine chromatin network. From this median thickening and at either end of the nucleus, one or two narrow, linear thickenings or ribs run in a longitudinal direction and they soon bifurcate. Near the nodes these ribs are connected by similar oblique and transverse thickenings, constituting Nageotte's marginal plexus.

The marginal plexus ends in a sort of protoplasmic collar around the node. The protoplasmic ribs of adjoining segments end in this collar. The collar represents a line of continuity between two nucleated segments of the schwannian syncytium and not a line of separation between two independent Schwann cells. Nageotte has shown that the rings revealed by Ranvier's application of silver nitrate to the fresh nerve have the same value as the black lines that outline the peritoneal endothelia, when treated similarly with silver nitrate. They mark a superficial boundary between two nucleated territories but they do not interrupt their deep continuity.

The medullated axone, then, is enclosed in a continuous and multinucleated syncytium provided with linear thickenings which anastomose in plexuses. The portion of the syncytium corresponding to an internodal segment possesses a single nucleus. It is this ensemble, the segment of the syncytium with its nucleus, that goes by the name of Schwann cell. The Schwann cell is a physiological unit but anatomically it is continuous with adjoining segments in an unbroken syncytium.

stifle them altogether, so that in the end a neurofibroma may become a more or less pure fibroma.

It must be conceded that the schwannian nature of the tumor cells has not been demonstrated clearly. The nuclei have been described accurately by many pathologists, but the features noted are not sufficiently characteristic to be regarded as specific and belonging only to schwannian nuclei. The features of the cytoplasm are wholly lacking in precision and one cannot see clearly how they differ from those of fibroblastic cytoplasm. From this point of view the opposition of certain pathologists to the neurectodermal hypothesis of encapsulated neurofibromas is justified.

Various circumstances have led me to support the schwannian conception of neurinoma. The friendship with which Professor Jean Nageotte has long honored me has permitted an intimate acquaintance with his work on the peripheral nerves and particularly on the schwannian syncytium, to which, following Held, he justly gives the name of peripheral neuroglia. Not only have I been able to verify all that Nageotte has written of the schwannian syncytium, but also I have been able to make new observations on experimental specimens that he prepared for me with the collaboration of Mlle. Guyon, and which I have treated by my own methods. Finally, certain exceptionally favorable specimens of human neurinomas and then all of the neurinomas in my collection, studied with these methods, have convinced me that these tumors are constituted almost exclusively of Schwann cells and that the connective-vascular tissue enters into their constitution to the same extent as it enters into the constitution of central gliomas, no more and no less.

In the present paper I shall describe the normal syncytium of Schwann according to Nageotte. I shall then consider its characteristics, when stimulated to autonomous proliferation and to the production of experimental schwannomas. In Part II I shall apply these observations to the study of human neurinoma.

ARTIFICIALLY STIMULATED PROLIFERATION OF THE SCHWANNIAN SYNCYTIIUM

Section of a medullated nerve is followed immediately by wallerian degeneration of its fibers. In the proximal stump the degeneration is limited to the tip; it extends through the entire length of the distal stump. Wherever it exists, the degeneration is accompanied (fourth day) by mitotic proliferation of the schwannian nuclei (Büngner). As a result of this proliferation, schwannian sprouts invade the wound both from the proximal and from the distal stump, and they approach each other and finally unite (Nageotte). The neurites of regeneration coming down from the upper stump travel in the corresponding schwannian sprouts; together they constitute Nageotte's neuroglioma. The purely schwannian sprouts coming up from the lower stump construct a schwannoma (Nageotte's peripheral glioma) which, after its union with the central neuroglioma, is in its turn invaded by the descending neurites. Guided by the schwannian bands, the neurites soon gain the blighted fibers of the distal stump and repopulate them. On the arrival of the neurites in the schwannian syncytium its proliferation ceases immediately.

EXPERIMENTAL SCHWANNOMAS NAGEOTTE'S EXPERIMENTS

If, as Nageotte has done, we prevent innervation of the distal stump by tearing out the proximal end of the nerve together with its roots, the peripheral schwannoma grows for a long time and its fibers hypertrophy. Reduced to their syncytium, the fibers of the distal stump hypertrophy also. We shall see that these hypertrophies correspond not only to a volumetric increase of the syncytium (Nageotte), but also to an amitotic proliferation of its nuclei.

Proliferation of the schwannian syncytia is still more pronounced if, after cutting the sciatic nerve of a rabbit and tearing out the proximal end, together with its roots and ganglia, an incision is made through the distal stump about 1 cm. below the first incision; the piece of nerve is left in place. In this way an isolated piece of nerve is deprived of its circulation temporarily (Nageotte). The fragment of the nerve shortens. From each end of its contracted perineurial casing there protrudes a hemispherical hernia consisting

ENDONEURIUM AND MEMBRANE OF SCHWANN

The endoneurium is generally accepted as a connective tissue, an assemblage of collagen and reticulin fibrils together with branching cells, all of mesodermal origin. I shall accept this postulate provisionally, although it is open to discussion. Two kinds of fibers go to make up the fibrillar endoneurium.

1. A system that I shall call interstitial, occupying the angular space where three medullated fibers meet. This interstitial system is composed of fibers running parallel with the nerve, branching, perhaps anastomosing with one another. Van Gieson's solution stains them faintly; aniline blue of the trichrome stain shows them clearly; silver methods impregnate them intensely. These interstitial argyrophil fibers correspond to the networks of Key and Retzius, and of Cajal. In their interstices are found the endoneurial cells, of spongy cytoplasm, elongated in the direction of the nerve, their branches anastomosing with those of adjoining cells.

2. A fibrillar sheath first demonstrated by Plenck and well described by Laidlaw. This sheath consists of delicate argyrophil fibrils, seen best after fixation in Zenker's fluid. The fibrils are circular, oblique and longitudinal. They anastomose to form a web or net which is applied closely to the Schwann cell. They are continuous with the longitudinal fibers of the interstitial endoneurium. In brief, the schwannian syncytium is enclosed in a web of reticulin resembling that which surrounds the epithelial lining of the intestinal glands, and, like the latter, it is a basement membrane.

When a cross-section of a nerve is impregnated by Laidlaw's silver method and counterstained by ponceau-acid fuchsin followed by light green, the result is very curious. Each nerve fiber is enclosed in a delicate black ring of reticulin which is closely applied to the schwannian cytoplasm. The cuticle of the membrane of Schwann is invisible. This should be taken in connection with Laidlaw's interesting demonstration in the roots of the spinal nerves, that the membrane of Schwann and the Plenck-Laidlaw argyrophil sheath accompany the root fibers into the marginal glia to the same point. These two structures have exactly the same distribution. They are closely connected, therefore, and seem even to be included one within the other. One may ask whether they are not one and the same thing. Presently we shall see the importance of this conception.

of those pieces that were excised after three months' growth, fixed in Bouin's picro-formol, embedded in paraffin and cut in serial sections, both transverse and longitudinal. The stains used were the trichrome (derived from Mallory's connective tissue stain); Laidlaw's silver stain for reticulin, either alone or followed by ponceau-acid fuchsin and light green; and Mallory's phosphotungstic acid hematoxylin.

Since similar pieces have already been studied by Nageotte, I shall again be obliged to draw on his descriptions. Those who, like me, have studied subjects already treated by him will soon learn how little there is left to glean after this great observer. It so happens, however, that the little which I am able to add to his observations is of decisive importance for the schwannian hypothesis of neurinoma.

THE DISTAL SEGMENT

With this name I designate the fragment of the distal stump that has been cut and left in place, as in Experiment I. This piece of nerve is relatively slender, having lost about one-fourth of its original caliber. Its fibers are degenerated and swollen here and there by fatty ovoids not yet absorbed. In the intervals between the ovoids the fibers have a uniform caliber; they are geometrically perfect cylinders. This does not mean that all fibers have the same diameter. Some are broad, some narrow. It is probable that the caliber of each degenerated fiber depends on its original caliber before degenerating.

On cross-section the fibers are seen to be notably broader than in the days immediately following wallerian degeneration; they have undergone Nageotte's secondary hypertrophy. The fibers are separated from one another by abundant endoneurial collagen, very dense and of sclerous aspect. Each fiber is surrounded by a dark blue, circular sheath (aniline blue of the trichrome stain) closely applied to it. At first sight this sheath appears to be homogeneous but it is really composed of longitudinal fibrils pressed closely against one another like the staves of a barrel. The fibrils may be detected in cross-sections where a defect in the knife has pulled them apart a little. In longitudinal sections they outline a blue striation on the surface of each nerve fiber.

With Laidlaw's silver method, these fibrils impregnate indis-

of endoneurium and the ends of the nerve fibers. On the conclusion of wallerian degeneration and revascularization of the endoneurium, these damaged nerve fibers, reduced to their syncytia, undergo gigantic hypertrophy. Schwannian sprouts emerge and, together with the regional connective tissue, construct a fibroglioma at each end of the fragment. The one, situated between the fragment and the distal stump, unites with a similar fibroglioma arising from this stump; together they constitute an aneuritic cicatrix very similar to an innervated cicatrix. The other fibroglioma, springing from the proximal end of the fragment, becomes an enormous tumor which invades the neighboring muscles and is still growing after five months.

Into this experiment, which I shall designate as Experiment I and which resembles one already made by Bethe, there may creep a source of error. Neurites coming down from the spinal orifices or elsewhere may repopulate some of the schwannian bands, myelinate them and create an illusion of autogenous regeneration. In another experiment, which I shall call Experiment II, Nageotte and Guyon prevented any intrusion of this kind. From the right sciatic nerve of a rabbit they excised a piece 1 cm. long and transplanted it alongside of the left sciatic nerve of the same animal, separating the gluteal muscles without cutting them. From each end of the graft there grew a schwannian tumor which adhered to the neighboring tissues. In three months' time such a tumor may have acquired twenty times the volume of the original graft and still be growing after five months.

These tumors are exactly like those observed after the simple section of the nerve in Experiment I. They demonstrate the remarkable fertility of the schwannian syncytium, when deprived of the control exercised by the neurites. Both tumors are of capital interest to us in that they enable us to fix precisely the characteristics and properties of the schwannian syncytium when in autonomous proliferation.

Nageotte and his collaborator, Mlle. Guyon, repeated these experiments with admirable results and presented me with some remarkable specimens which required only to be cut, stained and studied. For any interest aroused by the present work, the reader is indebted to them; if it should be judged mediocre or worse, it is to me alone that criticism should be directed. I shall make use only

medullated fiber; but each territory corresponding to a former inter-nodal segment now possesses several nuclei and several stellate cytoplasmic bodies, anastomosing with one another and with those of adjoining segments which have multiplied similarly. Since the axis cylinder and the myelin sheath have disappeared, the schwannian syncytium occupies the entire thickness of the fiber and not merely the periphery. It is cortical only around the fatty ovoids.

To these observations, which conform exactly to those made by Nageotte, I should add another. The schwannian nuclei of the hypertrophied syncytium are more numerous than those of the fiber when recently degenerated and reduced to a filiform band. The nuclei often present longitudinal fissures of varying depth; or two or three elongated nuclei are found side by side in close contact in the same cytoplasm. It is obvious that these groups result from amitotic division and longitudinal cleavage. At other points the nuclei, still near one another, are ranged in an oblique line but in different axial planes of the initial cylinder, as if the nuclei of an isogenic group had slipped past one another, together with their respective cytoplasms, and were spacing themselves regularly along the degenerated fiber. This disposition is rare in the distal stump but becomes more frequent near the cut end of the nerve. I shall return to it later. This proliferation in the heart of a nerve that is unable to elongate is a curious phenomenon. It explains the secondary hypertrophic broadening of Büngner's bands, a broadening not due solely to volumetric increase of the Schwann cells but also to their amitotic multiplication, to their hyperplasia.

SEGMENT (EXPERIMENT I) OR GRAFT (EXPERIMENT II)

The events in these two pieces are essentially the same. For purposes of description either may be chosen. I have already described the shortening of the piece of nerve when simply isolated (Experiment I), or when isolated and then transplanted (Experiment II). I have noted also the hernia of its contents, the endoneurium and nerve fibers, protruding beyond the divided perineurium. An edema infiltrates the entire piece, affecting both its endoneurial contents and the perineurium, the collagenous laminae and endothelia of which separate, leaving between them large lacunae filled with an absolutely colorless fluid. By isolating the elements of the nerve, the edema enables us to follow them more easily in serial

tinctly. They are violet and not black. They run almost exclusively in a longitudinal direction and are much coarser than the argyrophil fibrils that run in all directions and envelop the normal nerve fiber as in a net or web. They are continuous with the interstitial fibers of the endoneurium. These latter fibers seem to be no more numerous than those of the normal endoneurium, but they are broader and their colorability with aniline blue and by the Van Gieson method has increased to the detriment of their argyrophilia. The endoneurial cells have undergone no appreciable change.

In a word, the entire endoneurial interstitial substance has become denser and collagenized. The original argyrophil reticulum has been replaced by a coarse, sclerous, collagenous network. It is fitting to recall here the remarkable property possessed by this fundamental substance of the sclerous endoneurium, of swelling in weak solutions of nitric acid. This property is confined to this tissue and to the collagen of the cornea (Nageotte).

The degenerated nerve fibers color pink in acid fuchsin. No fuchsinophil membrane comparable to the primitive membrane of Schwann separates them from the collagenous sheath. It seems as if the latter, which has replaced Laidlaw's reticulin membrane, has at the same time replaced the membrane of Schwann. Is not this an argument in favor of the idea that the reticulin membrane and the membrane of Schwann are one and the same thing?

In cross-sections the degenerated fibers enclosed in their collagen tubes seem to consist of two substances, a homogeneous fluid faintly colorable by fuchsin and tiny angular bodies of various sizes, some punctiform, others larger and staining more deeply. In some of the larger bodies there is seen a round nucleus. In longitudinal sections there are protoplasmic bands regularly calibrated and set with many oval elongated nuclei with delicate reticular chromatin and often containing a large plasmosome. The cytoplasm appears to be striated longitudinally. In reality this striation corresponds to the juxtaposition and to the fasciculation of slightly sinuous cell bodies, together with their branches running lengthwise along Büngner's bands. Conforming to Nageotte's conception, a Büngner band consists of stellate cells with filiform prolongations anastomosing in a network, the meshes of which are drawn out lengthwise.

In brief, this syncytium reproduces the essential reticular and anastomotic arrangement of the schwannian syncytium of the

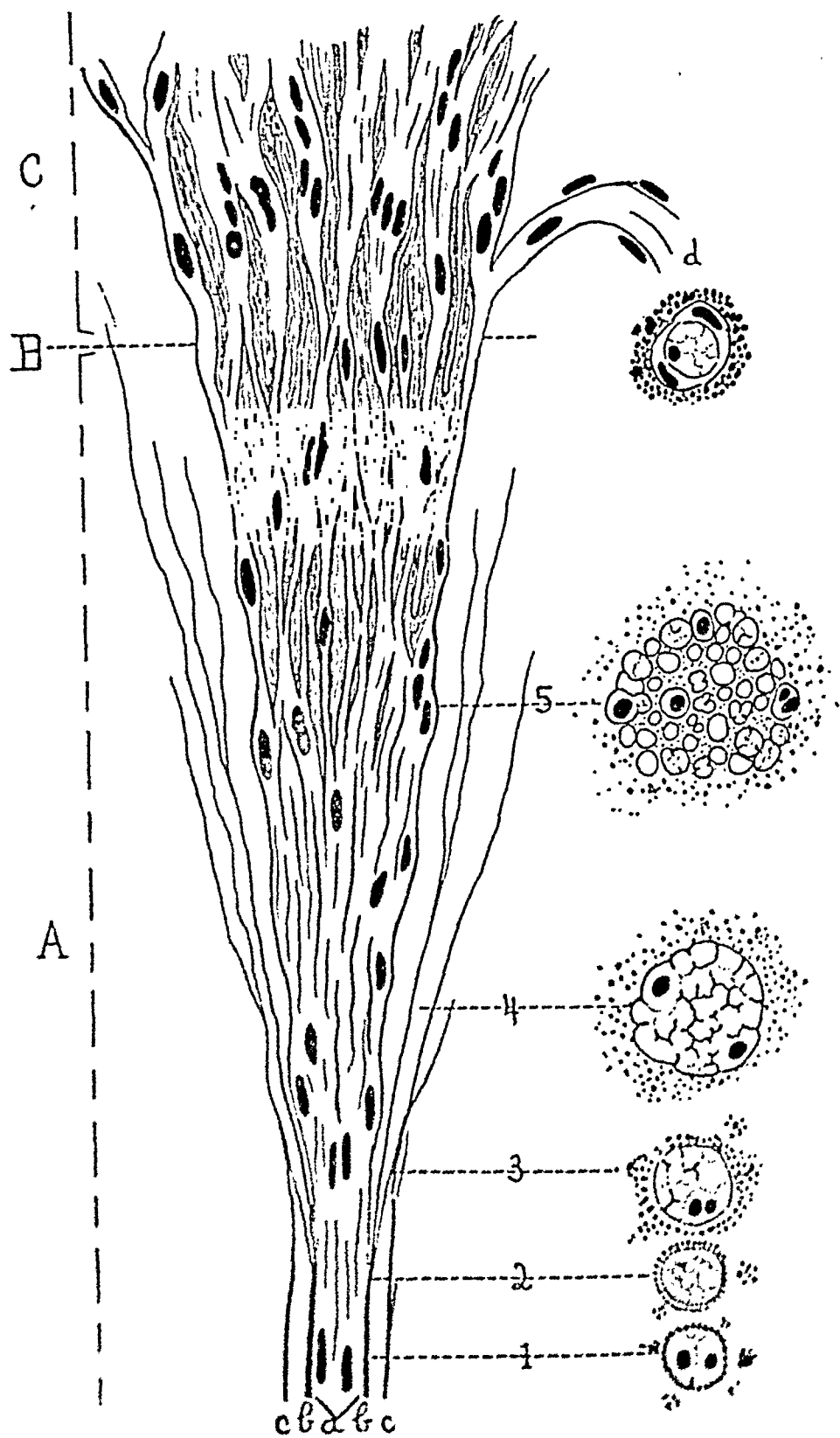


DIAGRAM 1

sections and greatly facilitates histological analysis. The edema is much more pronounced in the segment that is simply cut and left in place (Figs. 1 and 3) than in the graft (Fig. 10). For this reason in this description I shall use chiefly the specimens of Experiment I. To avoid prolonging this paper beyond measure, I shall limit myself to a synthetic statement: for analysis the reader is referred to the figures and to their copious legends.

The phenomena that I am about to describe begin at each end of the incised segment and extend progressively to the middle of it, so that by studying first the nerve fibers and the endoneurium in the middle of the segment and then toward either end, we may reconstitute the successive stages of their transformation.

THE SCHWANNIAN BANDS (Diagram)

Secondary hypertrophy of the degenerated fibers, already distinct in the distal stump, assumes enormous proportions in the incised segment. If we should designate the mean diameter of the distal bands by the figure 1, their diameter in the middle of the segment would be from 4 to 10 (Fig. 1) and near the ends from 10 to 20 (Fig. 2). This broadening of the original fiber is accompanied by active amitotic proliferation of its nuclei. It is followed by longitudinal cleavage which converts each schwannian band into a bundle identical with the innervated bundle of regeneration, except that it possesses neither neurites nor myelin.

We shall follow the steps of this transformation. Our point of departure will be the reticular syncytium enclosed in a thick collagenous sheath formed of coarse, closely-set, longitudinal fibers which have replaced the former membrane of Schwann and the Plenk-Laidlaw reticular sheath.

The schwannian cytoplasm fills the collagen sheath completely; at this point there is no fluid between the tissue elements. The cytoplasm is pale pink, vitreous and homogeneous. Cross-sections (Fig. 1) show it to be divided into polygonal territories of unequal dimensions by very thin septa of darker red color. The septa are dotted with tiny points still darker red; these are cross-sections of delicate fibrils. Here and there a slight retraction, a shrinking of certain territories, shows that each of them is bordered by a thin membranous condensation. It is obvious that the protoplasmic septa between contiguous territories are very thin membranes, each

vicinity of the nuclei. These nuclei are elongated ovals, sometimes gigantic, containing a large plasmosoma, often fissured longitudinally. Isogenic groups of two or three nuclei are often seen.

Around these broadened fibers the collagenous sheath is distended. Its longitudinal fibers are no longer contiguous, but separated from one another by the swollen contents. Fine collagen and argyrophil (Laidlaw's method) fibrils soon appear in the septa. Some of them are continuous with fibers of the perineurial sheath; others are axial without visible continuity with any preformed collagen. Still other fibrils are added to the first group and little by little each cytoplasmic septum (red) is replaced by a collagenous septum (blue). The partitioning is more precocious at the periphery of each fiber than in its center, so that at a certain stage a cross-section of a fiber gives the appearance of peripheral spurs projecting inward. The spurs are the beginnings of radial septa.

When the collagenization of the membranes of Schwann is finished, each cell (nucleated body together with its multiple and anastomosing branches) of the syncytium is enclosed in a continuous tubular sheath, retiform like the cell and moulded exactly on it (Figs. 2, 3 and 4). Later the party walls split so that there comes to be an individual sheath around each fiber (Figs. 2, 6 and 8). In this way two cytoplasmic branches, locally distinct but contiguous, may separate from each other, each enclosed in its collagen sheath, which nevertheless remains connected with its fellows by fine blue fibrils. Local isolation ceases at the first anastomosis, where two cytoplasmic cylinders unite in a single fiber within a single tubular collagenous sheath. Since collagenous partitioning always follows cytoplasmic partitioning it is always more advanced, more precocious around the cellular prolongations than around the nuclei. It appears last between the nuclei of the same isogenic group.

All of these phenomena begin and proceed in the degenerated fiber, even when it still contains fatty ovoids (Figs. 2, 4 and 8). They are seen even alongside of the ovoids themselves (Fig. 2), the presence of which does not interrupt the continuity of the schwannian syncytium.

In this way the schwannian syncytium of each degenerated fiber is converted into a retiform bundle exactly like an innervated bundle of regeneration before myelinization. This is not exactly what Nagotte observed. In his experimental material the col-

of them closely adherent to its cytoplasmic territory but susceptible of separation. These are the membranes of Schwann, forming around each of the territories that have arisen from a single Schwann cell. The broader territories often contain a nucleus. Here and there are cross-sections of several nuclei, two, three or more in the same fiber.

In longitudinal sections (Fig. 3) these fibers appear to be striated. The striation corresponds to different shades of color of the cytoplasm that are elongated in the direction of the fiber, of the membranous septa which separate them, and of the tiny fuchsinophil fibrils lodged in the septa.* The striation ceases or diminishes in the

* After fixation in picro-formol, phosphotungstic acid hematoxylin stains these fibrils and these septa bluish. It is probable that the fibrils correspond to those which Nageotte demonstrated with Benda's neuroglia stain.

DIAGRAM I

Experiment II. Transformation of a degenerated fiber into an aneuritic bundle of regeneration.

(A) Incised segment. (B) Limit of the hernia. (a) Degenerated medullated fiber reduced to its schwannian syncytium and hypertrophied secondarily. (b) Its collagen sheath. (c) Interstitial endoneurium dissociated by edema.

(1 to 5) Successive aspects of the fiber at different levels. On one side a longitudinal section; on the other a cross-section.

(1) Two isogenic nuclei. Incomplete cytoplasmic partitioning. The collagen sheath is a little distended by its contents. Its longitudinal fibrils are slightly separated from one another.

(2) Internuclear region. Cytoplasmic partitioning (dots in cross-section, delicate lines in longitudinal section) is more advanced here than in the region of the nuclei. The laminae of the collagen sheath begin to separate.

(3) Beginning of collagenous partitioning. The old collagen sheath of longitudinal fibers has separated from the schwannian cylinder and is being incorporated into the interstitial endoneurium. The cylinder itself is covered by a new reticular sheath, chiefly of circular fibrils (dots in longitudinal section) which gradually grows more dense.

(4) Collagenous partitioning is being completed, the collagen replacing the cytoplasmic septa.

(5) Cleavage of the collagenous septa and isolation of the young cylinders. Between them is the neo-endoneurium, the framework of which is constituted by longitudinal fibrils liberated from the septa at the time of their cleavage.

(C) Infiltrating schwannoma. Cylinders continuous with those of an aneuritic bundle invading the connective tissue. Isogenic nuclear groups on the same transverse plane or in process of shifting along the cylinder.

(d) Schwannian cylinder in the stage of cytoplasmic partitioning invading the connective tissue. It passes through the same stage of partitioning as the original cylinder. It is separated from the surrounding tissue by an endotheliform perineurium, formed probably by cells emigrated from the endoneurium of the incised segment and not by local connective tissue cells.

creases progressively and the collagen fibers, increased in number and deprived of their jelly, resume their habitual precision.

The gelatinous envelope surrounds each schwannian fiber exactly, constituting a sheath system. Between adjoining gelatinous sheaths there are clefts occupied by endoneurial cells and by a network of their branches. These cells have multiplied by amitosis. When the jelly has disappeared, these cells install themselves in its place between the liberated and multiplied fibers. Of the two types of cell present, the endoneurial cell and the schwannian syncytium, which seems to have the decisive influence on the production and on the increase of collagen? Everything favors the schwannian syncytium.

If we now examine attentively the Laidlaw sheaths which have become collagenized, we see that certain of them are reduplicated and that others are partially separated from the schwannian fiber, the cytoplasm of which is in contact with the gelatinous substance. This denudation of the syncytium lasts but a short time. A collagenous pellicle, consisting in reality of a delicate web of fibrils, especially circular fibrils, identical with the Plenk-Laidlaw sheath of the normal fiber, is being constructed and is replacing the initial cytoplasmic membrane. Thus the syncytium is soon enclosed in a new and very thin sheath which soon thickens in its turn (Fig. 11).

In brief, at the time when the old collagenous sheath is separating from the syncytium and becoming incorporated in the interstitial endoneurium, a new reticular sheath is being produced on the surface of the syncytium. The degenerated fiber seems to undergo a sort of moulting like that of the imago when it issues from the chrysalis. It is at this time that the internal collagenous septa begin to appear, those septa that convert the degenerated fiber into an aneuritic schwannian bundle. Thus the entire sheath system of the bundle of regeneration and even the interstitial framework of the endoneurium seem to be determined by the schwannian syncytium. Nageotte has said that the schwannian syncytium constructs the nerve and the neurites occupy it. We may add, I believe, that the schwannian syncytium constructs the endoneurial collagenous framework and the endoneurial cells occupy it.

THE INFILTRATING SCHWANNOMAS

Within the distal stump, as in the cut segment of the nerve, schwannian proliferation is moderate in degree (Figs. 5, 6, 7). Each

lagenous partitioning was roughly sketched but it remained incomplete, never advancing to complete cleavage. Nageotte held that complete cleavage is peculiar to innervated bundles and that it was determined by the intrusion of the neurites. My own observations show that the influence of the neurite is not indispensable. This is of capital importance for the understanding of neurinomas.

The collagen which forms in the place of, and without doubt at the expense of, the schwannian membranous septa without the intervention of any mesodermal cell, is an endoneurium — a neo-endoneurium — the endoneurium of an aneuritic bundle. It is exactly like the neo-endoneurium of an innervated bundle of regeneration. Like the latter it is invaded secondarily and occupied by typical endoneurial cells, but these cells have not created it.

EVOLUTION OF THE OLD ENDONEURIUM THE NEO-ENDONEURIUM

As I have already said, all of the phenomena just described begin inside of the original, thickened Plenk-Laidlaw sheath. This disappears as a sheath in a remarkable manner. In regions where the degenerated and hypertrophied fibers have not yet undergone collagenous cleavage (Figs. 1 and 11), each of them still enclosed in its collagenous sheath is surrounded by a sort of casing of gelatinous appearance, composed of an extremely delicate network, the fibers of which are non-argyrophil and stain but faintly with aniline blue. This casing encloses not only the collagenous sheath of the degenerated fiber but also a group of interstitial endoneurial fibers. These latter, which appear to be voluminous and deeply stained under low magnification, consist of numerous fibrils associated in bundles. The contours of the bundles are indistinct. They seem to dissolve into the gelatinous substance. A little reflection will show that this apparent jellification of the fibers is inadmissible. If they had swollen before disappearing, their colorability and their number would have diminished. On the contrary, their number has increased and their colorability is intensified. There is rather the impression that new fibers are forming little by little around the pre-existing endoneurial fibers by condensation of the gelatinous substance which bathes them. The new fibers join the old ones to form the bundles. That which tends to confirm this idea is that as one approaches the cut end of the nerve the gelatinous substance de-

Around the smaller bundles there is a single layer; around the larger bundles they are stratified. Nageotte says that this newly formed perineurium is constructed by connective tissue cells from the invaded region which is adapting itself to its schwannian guest. For reasons which it will be more opportune to develop later, I have adopted a different opinion. For me, the endotheliform cells are cells which have emigrated from the endoneurium of the incised segments; they have followed the schwannian sprouts and construct protective sheaths for them.

I shall add a final observation. The perineurium of the degenerated nerve, delaminated, edematous, converted into a lacunar sheath, is invaded by many sprouts. These sprouts push into an optically empty fluid as free from albumin as is the cerebrospinal fluid. The sprouts are always enclosed in a reticular sheath reinforced by longitudinal collagen fibrils. Secondarily and later the endoneurial cells emigrate, and arrange themselves around the sprouts as miniature perineuria. I insist that the collagenous sheath of the sprout, the rudiment of its endoneurium, is constructed before any contact with an endoneurial cell, and under the sole influence of the schwannian syncytium (Fig. 7).

SUMMARY OF PART I

The schwannian syncytium enters on mitotic proliferation (Büngner) when the medullated axone which inhabits it has been separated from its trophic center and degenerates. The new nucleated territories conserve their syncytial relation. From the cut ends they invade the wound until the neurites, coming down from the proximal stump, penetrate them and together with them construct the bundles of regeneration.

If innervation is prevented, the proliferation of the schwannian syncytia continues both inside of the nerve and from the cut end. It becomes exuberant when the distal stump is cut a short distance below the first incision (Nageotte). The isolated piece of nerve gives rise to a voluminous, infiltrating, schwannian tumor.

Study of the syncytium in proliferation, both in the nerve trunks themselves and in the surrounding connective tissue, shows that each Schwann cell is capable of being transformed into an aneuritic bundle of regeneration, purely schwannian, retiform, and morphologically identical with the innervated bundles before their myelin-

degenerated nerve fiber is transformed into an aneuritic bundle of regeneration broader than the primitive fiber which it has replaced. Usually at the end of three months the transformation is complete only near the cut ends. When the cut segment is very short, the change may involve the entire length of the degenerated fibers. However, in the original nerve the old fibers and the bundles derived from them do not elongate, neither do they form anastomoses.

At the ends of the cut segment there is a very different picture. From the cut surface there escapes a sheaf of aneuritic sprouts, continuations of the elements of the bundles of regeneration, which invade the surrounding connective tissue, anastomose with one another, reunite in bundles which in their turn soon ramify and join neighboring ramifications to form new bundles, and so on. Thus is constituted a peripheral fibrogloma of retiform or plexiform structure.

I shall dwell no longer on the general aspect of these schwannomas, which have been described so well by Nageotte, but only on certain features of their aneuritic fibers which alone interest us here. Some of the cellular bands which have long since invaded the connective tissue push no further. They are exactly like those of the incised segment. Cylindrical, enclosed in a thick sheath of collagen, they consist of a schwannian syncytium of elongated cells which do not fill the sheath completely. Between the cells and their processes there is more or less abundant fluid. Other bands are in active growth (Fig. 5); they consist of turgid cells that fill their very thin, reticular, collagenous sheath completely. Their nuclei are dividing, always amitotically. The isogenic groups of nuclei are being scattered rapidly by shifting and spacing of the nuclei along the fiber.

Beyond any doubt, the retiform structure is often constructed by lateral anastomosis of the advancing bands, as observed by Nageotte (Fig. 5); but it results also from longitudinal cytoplasmic partitioning followed by collagenous partitioning and then cleavage of the collagenous septa, exactly as I have described in the degenerated fibers which undergo hyperplasia in the incised segment. In other words, the invading bands are converted into aneuritic bundles (Fig. 6).

The isolated bands, the smaller and larger bundles, are always separated from the connective tissue by a more or less continuous layer of endotheliiform cells, constituting miniature perineuria.

gelatinous sheaths there are fissures occupied by proliferated endoneurial cells and by their anastomosing prolongations. In this jelly the collagenous fibers increase in number; those of the old Plenk-Laidlaw sheath exfoliate (*d*); then they separate little by little from the schwannian bundle and become incorporated in the interstitial endoneurium.

At (*b''*) the collagenous partitions isolate several schwannian prolongations from their fellows.

FIG. 2. Experiment I. Segment resected and left in place. Cross-section of hernia. Trichrome stain with aniline blue.

The magnification of this drawing is $2\frac{1}{2}$ times less than that of Fig. 1. The aneuritic bundles of regeneration here represented are continuous with the degenerated fibers of Fig. 1, but they are two or three times broader.

Below and to the right (*a*) the collagenous partitioning of each schwannian bundle is less advanced than at the left, where it is complete (*b*) and at (*c*) where cleavage of the partitions is beginning. Three bundles still contain fatty ovoids (*d*).

(*e*) Bundles incompletely partitioned and cut almost longitudinally. The interstitial endoneurium is edematous but rich in collagen fibers. Some of them have belonged to the original Plenk-Laidlaw sheath; they have become collagenized and they have separated from the schwannian bundles. There is no longer a gelatinous casing around the bundles. Each bundle, together with its constituent elements, is surrounded by a new Plenk-Laidlaw sheath already thickened and collagenized.

(*f*) Split bundle, the elements of which begin to disperse.

(*g*) Epineurial connective tissue invaded by schwannian bands.

FIG. 3. Experiment I. Segment resected and left in place. Longitudinal section supposed to be from between the cross-sections represented in Figs. 1 and 2; this of course is from another block. The degenerated and slightly sinuous hypertrophied fibers are visible for only part of their length.

(*a*) The axis of this fiber is crowded with fat drops, residue of myelin. The margins of the syncytium are set with rows of nuclei. From one side to the other transverse anastomoses maintain the continuity of the syncytium in all directions. At the left the section, becoming tangential, shows the incomplete transverse separation of the nucleated territories and their elongation in the direction of the fiber. At the left are seen the longitudinal collagen fibers which surround the schwannian syncytium (new Plenk-Laidlaw sheath).

(*b*) On the right a striated appearance. The lighter bands correspond to the polygonal territories of Fig. 1; the darker lines correspond to the partitions seen in optical section and to their non-collagenized fibrils (*cf.* Fig. 1). On the left the same schwannian band; isolated in its substance is seen a delicate blue collagen fibril.

(*c*) Schwannian bands cut obliquely and distended by enormous fat drops. Delicate anastomoses connect the nucleated territories.

(*d*) Schwannian band cut in two places. On the right an iogenic group of nuclei beside a fat droplet. Immediately to their left collagenous partitions separate the prolongations of the cells; the partitions respect the anastomoses and ensheath them. The crossed blue fibers are endoneurial collagen fibers placed above the schwannian bundle. On the left the same band presents a swelling containing a gigantic nucleus.

ization. This transformation is accomplished (*a*) by repeated amitotic division, (*b*) by longitudinal cytoplasmic cleavage, and followed (*c*) by the production of tubular, ensheathing septa formed of reticular collagen.

The production of the ensheathing collagen and of the entire interstitial endoneurium of the bundle is determined by the schwannian syncytium. The rôle played by the endoneurial cells is distant and indiscernible. It is only after the endoneurial framework has been constructed that the endoneurial cells come to occupy it.

The schwannian bundles that invade the ordinary connective tissue are separated from it by an endotheliform perineurium, formed in all probability not by the common connective tissue cells of the neighborhood, but by cells emigrating from the endoneurium of the degenerated nerves and being transformed into endotheliform cells.

DESCRIPTION OF PLATES

PLATE 65

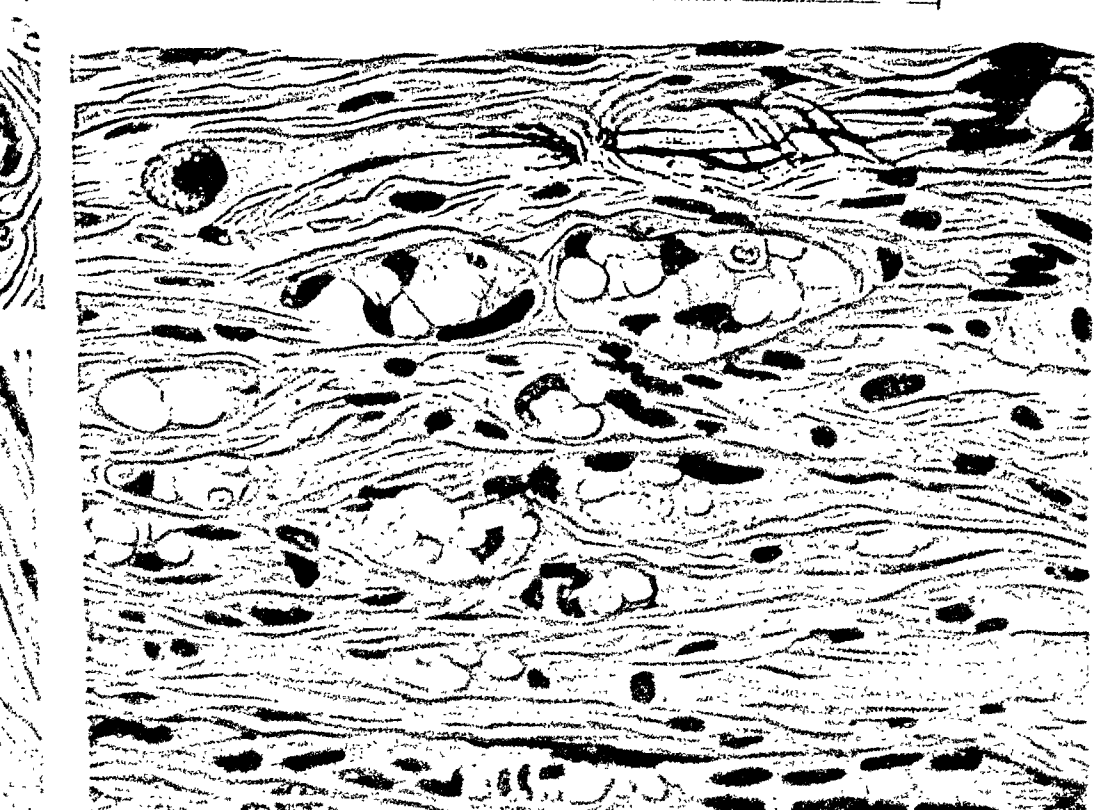
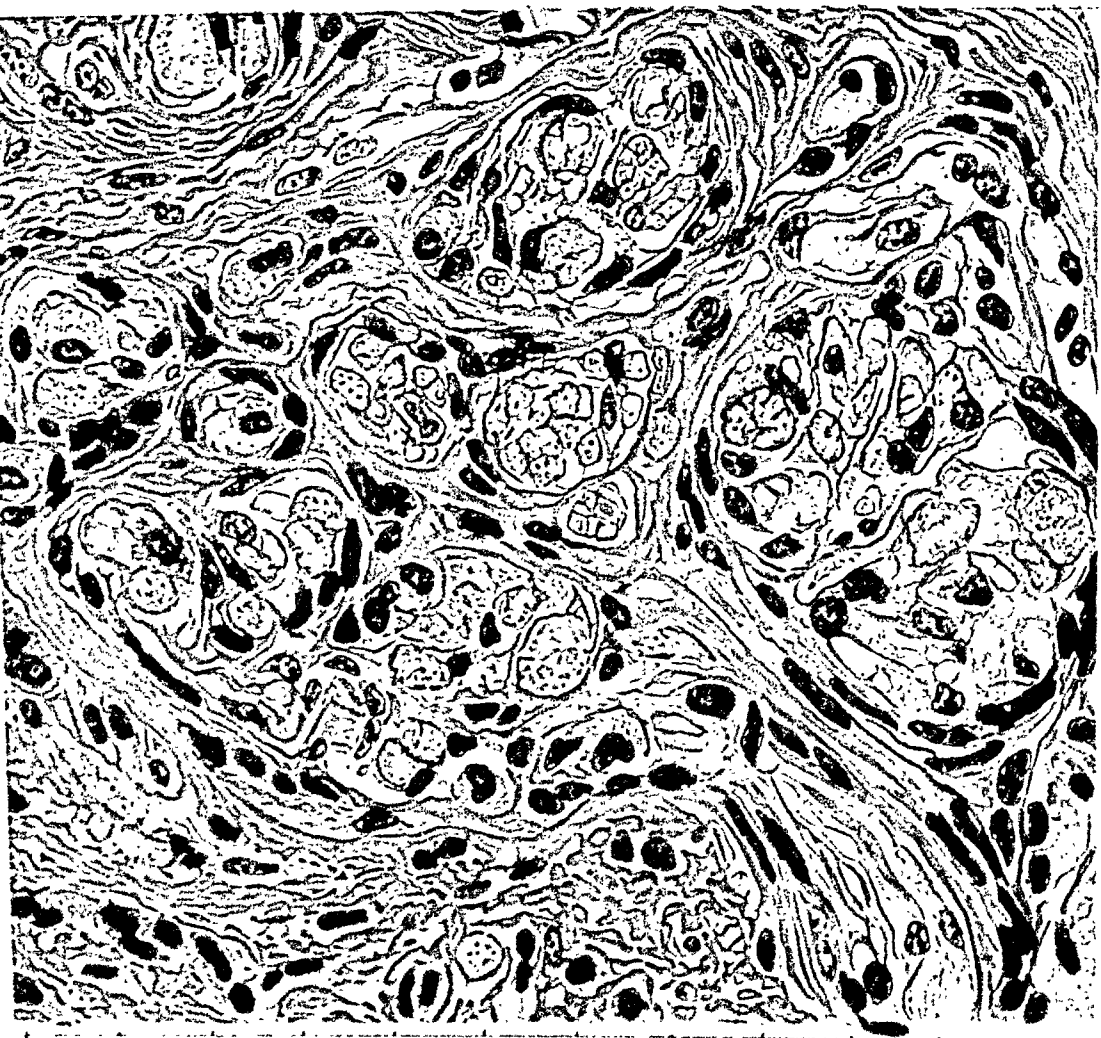
FIG. 1. Experiment I. Segment of nerve resected and left in place. Cross-section of middle. Trichrome stain with aniline blue.

The degenerated nerve fibers appear as pink circles mounted in blue (Plenk-Laidlaw sheath sclerosed). Their size varies greatly. Some (*a*) are scarcely broader than the largest fibers of the distal stump; others are four times as large (*b*).

All of the nerve fibers are divided into polygonal territories by septa dotted with dark red points (fibrils). Slight shrinking of some of the territories shows that in reality the septa are boundary membranes (membranes of Schwann). Some territories broader than the rest contain a nucleus. Some fibers present two nuclei (isogenic group) in the same transverse plane. In brief, each degenerated and secondarily hypertrophied fiber is converted into a schwannian bundle, composed of nucleated cell bodies and their multiple and anastomotic prolongations, all elongated in the direction of the nerve and crowded into the old Plenk-Laidlaw sheath (*b*), which has become collagenized. The fibers of this sheath, spread apart by the increase of the schwannian syncytium, are no longer contiguous as in the distal stump; they appear as blue dots.

Collagenous fibrils and then collagenous septa begin to replace the protoplasmic membranes of Schwann (*b'*). At (*b''*) the schwannian bundle, deprived locally of its sclerosed collagenous sheath, is enclosed in a thin collagenous pellicle of new formation and contemporaneous with the fibrils that begin to appear in the internal cytoplasmic septa.

Each degenerated fiber is enclosed in a thick, pale blue, gelatinous sheath which surrounds not only the Plenk-Laidlaw sheath, but also a group of collagenous fibers of the interstitial endoneurium (*c i*). Between the



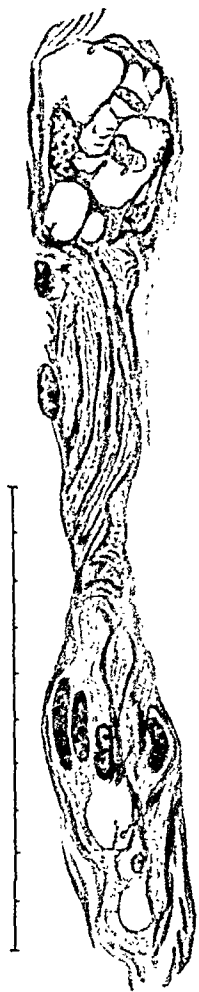
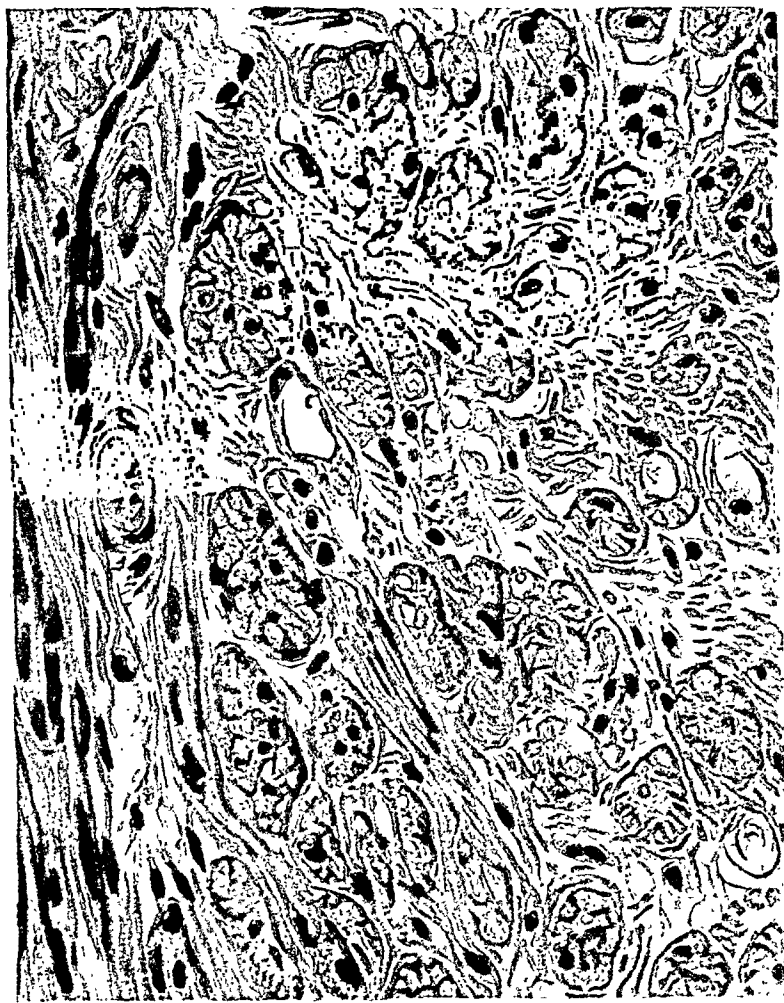
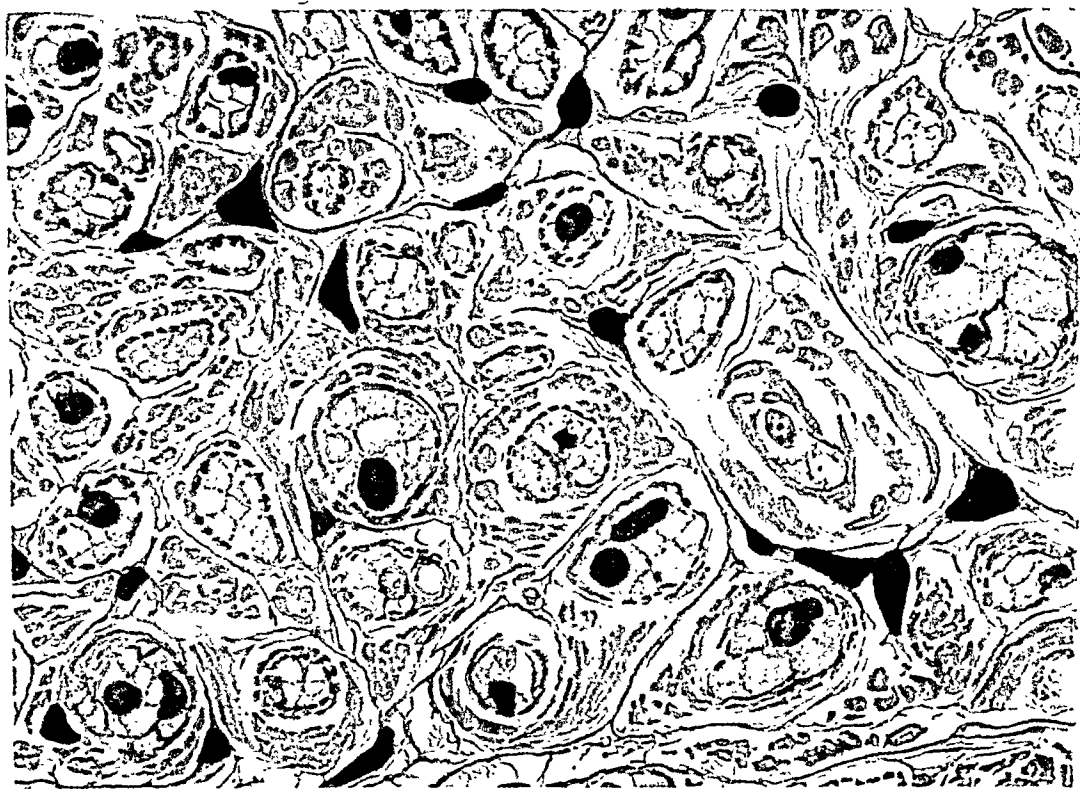


PLATE 66

FIG. 8. Aneuritic bundle of regeneration from the margin of the endoneurial hernia (Experiment I).

Below, swelling of the old schwannian band containing fat droplets. The bundle has escaped from the nerve. Its collagenous cleavage is finished and the young sprouts invade the surrounding connective tissue, assuming successively the aspects of Figs. 5 and 6.

FIG. 9. Another bundle near that of Fig. 8.

In the lower half note the group of isogenic nuclei that have remained in the same transverse plane and form a palisade. The nuclei are flanked above and below by a collagenous fibrillar layer, very dense, composed of the collagenous sheaths which enclose each of the cellular prolongations above and below. They constitute a collagenous palisade on each side of the nuclear palisade.

In the middle, a schwannian band of the same bundle, in process of elongation, shows four isogenic nuclei which have ranged themselves in a row. On the right and left, other bundles are cut obliquely.

FIG. 10. Experiment I. Graft of sciatic nerve near the end. Images similar to those of Figs. 1 and 2. The endoneurial edema is much less pronounced. The degenerated fibers show all the stages of longitudinal cleavage and collagenous partitioning.

FIG. 11. Experiment I. Segment resected and left in place. Middle region.

Two regenerated and hypertrophied fibers, freed from their old collagenous sheath, show in places their newly formed Plenk-Laidlaw sheath, either as delicate circular fibrils (curve of the central fiber) in tangential sections, or as dots in longitudinal sections (central fiber and fiber to the left and above).

FIG. 4. Segment resected and left in place. Same preparation as Fig. 3. A degenerated fiber converted into an aneuritic bundle between two fatty ovoids.

(a) Isogenic group of nuclei; on the left not yet separated by collagenous septa; on the right so separated.

(b) Cellular prolongations partially separated by septa which cease in region (c) where there is a large ovoid. The partitioning therefore is more precocious between the prolongations than between the nuclei.

(d) Collagen fibrils of the new Plenck-Laidlaw sheath.

(e) Remains of the gelatinous sheath.

FIG. 5. Experimental schwannoma. Experiment II. Young schwannian bands invading the connective tissue. Note their retiform arrangement. All of them are enclosed in a thin collagenous sheath which silver staining shows to be finely reticulated. At (a) the bands show distinct striation between the nuclei. The nuclei are dividing by amitosis. At (b) they form isogenic groups, rapidly dispersed owing to their shifting along the fiber which is elongating actively.

FIG. 6. Experimental schwannoma. Experiment II. Schwannoma invading the connective tissue. Bands older than those of Fig. 5.

This figure shows the stages of cleavage of the schwannian bands.

(a) Their transformation into bundles, at first protoplasmic.

(b) Their progressive collagenous partitioning. The collagen appears in the center of the band as well as along its borders where it has the appearance of spur-like processes.

(c) Cleavage of the collagenous septa followed by separation of the bands.

(d) Broadening of the bands.

The figure shows the progressive ensheathing of the bands and of the bundles by flattened cells which separate them from the connective tissue. These miniature perineuria spring perhaps from the regional connective tissue, adapting itself to the schwannian bands (Nageotte). However, I make every reservation in this interpretation. I believe that the new perineurium is formed of endoneurial cells of the incised nerve, which have followed the schwannian sprouts.

FIG. 7. Experiment I. Invasion of the edematous perineurium of the distal segment by a schwannian band.

The syncytium consists of very long cells with slender prolongations, highly retractile, bathed in a colorless fluid.

(a) Thin, pale perineurial cells. Cell (a) is not in contact with the schwannian band but on a lower level. The framework is surrounded by its reticular collagenous sheath (b), visible as a blue line bordering the band, or as a light blue veil at (b') (tangential section).

(c) Mobile cells from the endoneurium which are beginning to lay down a perineurial sheath. This sheath is discontinuous and very recent. The collagenous sheath exists before the arrival of these cells. It is determined by the schwannian band.

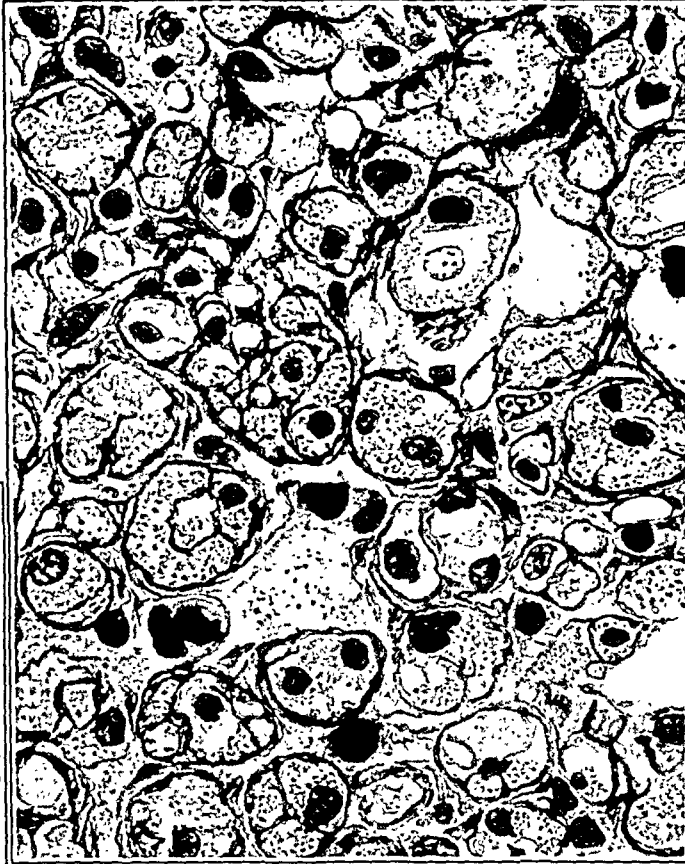




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11

THE TUMOR TISSUE. CLASSIC DATA

Among neurinomas, Antoni distinguished two structural types, Type A, fasciculated and clearly polarized, that is, arranged in elongated bands, and Type B, reticular, without polarity. Type A often presents palisading, the description of which is usually ascribed to Verocay; but it was noted before Verocay by von Hippel, Mayer, Francini, Antoni and others. The palisading is inconstant but absolutely pathognomonic when present. Type B, the reticular type, is constant. Probably it arises by dedifferentiation of Type A. It is characterized by hyaline degeneration of the intercellular substance. In short, despite its three possible aspects, fasciculated, palisaded and reticular, the neurinomatous tissue is a unity. The palisading is a complication of the bundles; the reticular is a degenerated form.

The basic substance is composed of delicate reticulated fibrils staining blue with Mallory's connective tissue stain (aniline blue), yellow or orange with Van Gieson's and black with Bielschowsky's method. When degenerated they become transparent like hyaline substance but softer, more like jelly. Neurofibrillar impregnation methods sometimes reveal a few neurites. It is improbable that these neurites form an integral part of the tumor; they are old neurites of the nerve trunk in which the tumor has grown.

This classification of the structural aspects of neurinomas seems to me to be very judicious. Like many of my predecessors I adopt it, not *a priori* but *a posteriori*, because my observations lead me to believe that it includes the essential facts; but the description of each tissue type, as one may read it in Antoni, is much too succinct. The same may be said of the innumerable subsequent descriptions. Each of them has brought to the support of the schwannian hypothesis of neurinomas a fresh confirmation of the structures seen by Antoni or some new structural details; but no one of them has assembled a group of arguments sufficiently convincing to rout the partisans of the fibroblastic hypothesis.

TECHNIQUE

It seems to me that this shortcoming is due to the universal use of the same techniques, first and foremost Van Gieson's. I shall not speak of the hematoxylin and eosin stain which should long ago

EXPERIMENTAL AND SPONTANEOUS SCHWANNOMAS (PERIPHERAL GLIOMAS)*

P. MASSON

(From the Laboratory of Pathological Anatomy, Université de Montréal, Montreal, Canada)

PART II

SPONTANEOUS SCHWANNOMAS

INTRODUCTION

The tumors to be discussed here are the encapsulated tumors of the peripheral nerves (neurinoma, lemmoma, peripheral glioma, neurofibroma and perineurial fibroblastoma) situated outside of the cranial cavity and outside of the spinal canal. I shall not touch on the encapsulated tumors of the cranial nerves or on those of the spinal nerve roots, not because these tumors are without interest or any different from the others, but simply because circumstances have not permitted me to accumulate a sufficient number of examples.

I shall take no account of the number of tumors in the same subject. The distinction between solitary and multiple tumors is purely clinical; it has no scientific value. A diagnosis of solitary tumor would have to be controlled by dissection of every nerve in the body; an accessible voluminous tumor may seem to be solitary, while others, smaller and situated more deeply, escape palpation. Furthermore I believe that solitary and multiple tumors are identical in structure.

THE CAPSULE

The characteristic element of these tumors, the capsule, enables us to eliminate from the discussion other tumors of the same nature, but infiltrating and diffuse. The capsule, formed of lamellae, envelops the tumor except at the point of attachment of the nerve. Here it is continuous with the perineurium and it probably represents the former perineurium distended locally by the growth of the tumor within it. This feature is important for it shows that the tumor is composed of one or several of the elements of the normal nerve enclosed within the perineurium.

* Received for publication March 18, 1932.

I. THE BUNDLES

The basic element of the bundle — the nucleated ribbon, the syncytial band — is in reality a nucleated cytoplasmic joist of circular cross-section. It is a cylinder, but a cylinder unequally calibrated and without free ends, being continuous with other cylinders. The cylinders are not only continuous longitudinally but they are also united laterally by oblique cylindrical anastomoses. Connected thus in all directions they form a net-like assemblage of three dimensions, the meshes being drawn out along the length of the fiber. The meshes are so elongated that the constituent cylinders are nearly parallel and so close to one another that they leave only long, narrow chinks, often mere potential spaces, between their surfaces of contact (Diagram 1).

THE CYLINDERS

Accurate cross-sections of a compact bundle show that the caliber of the cylinders is quite variable and that their structure is not less so. In general the caliber and the structural complication are proportionate (Fig. 3). By separating them the edema* makes their study easy in serial sections and permits of facile reconstructions (Figs. 1 and 2).

A SIMPLE CYLINDER

First of all I shall describe an elementary cylinder, as simple as one as possible, disregarding its connections. As a center I shall assign it a nucleus and for ends an imaginary transverse plane cutting it on either side of the nucleus halfway to the next nuclei of the same cylinder (Fig. 2 (g)).

(A) *Trichrome Blue*: (Fig. 2). The cylinder is not geometrically perfect. At the level of the nucleus it is enlarged, its diameter being about 7 microns, whereas near the ends, especially if it is very long (30 to 40 microns), it measures barely 2 to 3 microns in diameter. Its cytoplasm is colored uniformly deep pink. The nucleus does not fill nearly the entire area of the cell as happens in spindle-cell sarcoma, but only one-third or one-fourth. The form of the nucleus

* I refer to a true edema, a recent acute edema such as I have observed in my neurinoma of the piate, and not the false edema characteristic of Antoni's Type B. The former reveals but leaves intact the structures that I am about to describe; the latter results from their slow degenerative destruction. It is not in Type B, therefore, that these structures should be sought.

have been relegated to a museum of antiquities. Many who have used Mallory's methods and silver impregnations of collagen would have seen decisive things, if instead of using these techniques separately they had used both of them on the same section. In my own studies I have used the trichromes, derived from Mallory's aniline blue method, either alone or combined with Laidlaw's silver stain for reticulin, after fixation in Bouin's picro-formol.

BASIC MATERIAL

My studies were greatly facilitated by one specimen, a circumscribed painful tumor that elevated the mucosa of the anterior region of the palate in a girl 17 years of age. In the course of several months this tumor had attained the size of a bean. Finally the mucosa over the tumor ulcerated and the tumor grew rapidly. Examination of a biopsy removed by Dr. Ph. Panneton of Montreal revealed a typical neurinoma with many bundles and beautiful palisading. Eight days later the tumor was extirpated and it has not recurred.

The biopsy had the peculiarity that, having been recently and superficially infected, it was infiltrated by an abundant edema which dissociated the elements of the neurinoma before they had time to degenerate appreciably. The tumor itself was still more edematous. Many of its cells had disappeared, permitting an easy study of the collagenous framework. The edema being recent, especially in the biopsy, and occurring in a neurinoma of rapid growth, it permitted an examination of the bundles and of the palisades in exceptionally favorable circumstances. Later I was able to discover the same features in all of the neurinomas examined.

Every neurinoma grows; its constituents multiply, enlarge and evolve. In order to understand them they must be studied not only as functions of three dimensions in space, but as a function of time. Obviously this latter factor will escape micrographic technique, but one can, I believe, deduce it from the histological aspects and introduce it into the argument with small chance of error. We shall study successively the essential elements, the bundles and the palisades (Type A); then the degenerated forms, that is to say, the sclerosis attributed to the intrusion of connective tissue, and finally the reticulated areas (Type B).

cylinders where it is visible or around all of them. As well put the same question for the normal nerve fibers where the visibility of the Plenk-Laidlaw web is far from constant and depends largely on the fixative employed. Picro-formol, which I have used in these observations, is less favorable for its exhibition than the Zenker fixation recommended by Laidlaw. In these picro-formol sections, then, there is no reason for surprise at the exceptional and merely local success of silver impregnation of the web which probably exists around each cylinder. Even when the web is impregnated, it does not seem to be a net with empty meshes; the meshes seem to be occupied by a thin pellicle staining by the light green or by the aniline blue.

RELATIONS OF THE ELEMENTARY CYLINDER TO THE INTERSTITIAL TISSUE

This sheath is remarkably adherent to the cylinder. The interstitial edema never separates them but the edema shows that, through its longitudinal fibrils, the sheath is continuous with the other longitudinal argyrophil and collagenous fibrils. These latter fibrils are more voluminous, brush-like, anastomosing, and situated in the interstices between the cylinders just as the fibrils of the interstitial endoneurium are situated between the medullated fibers of a nerve (Fig. 8). The analogy between these fibrils and those of the endoneurium is completed by the presence in their interstices of a few branching cells with filiform processes and spongy cytoplasm. These cells are absolutely naked; they have no collagenous or reticular sheath. A few argyrophil fibers run alongside of them but there is lacking that intimate relation which binds cylinder and sheath. This is true not only of the simple cylinders just described but also of the others. It is unnecessary to repeat the description.

MORE COMPLEX CYLINDERS

The caliber of these cylinders is still more variable than of those just described, being from 3 to 10 microns at the ends and from 7 to 50 or 60 microns at the nucleus.

(A) *Trichrome Blue*: (Figs. 1 and 2). The cytoplasm has the same features as in the simple cylinders. The nuclei are often fissured longitudinally or split into two or three. They may remain in the same transverse plane (Figs. 2 and 3, broad cylinders) or they

is a long oval, often irregular, broader at one end than the other. Often it is fissured. Its scanty chromatin is arranged in a delicate marginal net. In the nuclear juice there are always one or more perfectly round and basophilic nucleoli. The nucleolus may be turgid and hollowed out by a large colorless vesicle which distends it.

The cylinder is never naked. It is always enclosed in a thin envelope staining blue with this technique and moulded exactly on the cylinder. In this sheath there may be distinguished very fine fibrils of nearly circular course and others a little more voluminous of longitudinal direction.

(B) *Van Gieson*: Using the powerful acid fuchsin of the National Aniline Co., of New York, the longitudinal fibrils show very well but not the circular ones. In cross-sections the protoplasmic cylinder seems to be stippled in red (cross-sections of longitudinal fibrils), the dots being united by a thin orange line.

(C) *Laidlaw's Silver Technique Followed by Ponceau-Acid Fuchsin and Light Green*: This gives important additional information (Fig. 3). The cytoplasm of the cylinder no longer has the homogeneous appearance described after trichrome staining (probably the mordanting action of the potassium permanganate). Its colorability by ponceau has partly disappeared. The only reds are the delicate lines which in cross-sections divide the cytoplasmic area into tiny polygonal territories and which in longitudinal sections trace a delicate striation. By changing the focus it is seen that the septa and the longitudinal markings are one and the same thing; they correspond to tubular septa which divide the initial cytoplasmic cylinder into many smaller cylinders incompletely separated from one another. At the ends of an elementary cylinder the partitioning is more or less perfect. In the nuclear swelling it is absent or imperfect; if present it is along the margin only.

In cross-sections the sheath enclosing the cylinder appears in general as a narrow green border dotted in black. Longitudinal sections show that the black dots correspond to cross-sections of the longitudinal fibrils just described as stained by aniline blue and picro-fuchsin. Exceptionally the silver impregnates a delicate web of reticulin (Fig. 11) visible in certain tangential sections, and it occupies exactly the place of the green sheath.

The resemblance of this web to the Plenck-Laidlaw endoneurial sheath is striking. We may ask whether it exists only around those

cylinder there is no essential difference except the degree of their complexity, and everything goes to show that this complexity is progressive — that it corresponds to evolutionary stages of the cylinders.

To recapitulate, the cylinder undergoes longitudinal partitioning, carried out first of all by tubular cytoplasmic membranes; it is completed first at a distance from the nuclei, finally reaching the nucleated zones. Later there occurs collagenization of these septa, then cleavage of the collagenous septa followed by their partial separation. Between the newly formed cylinders some of the longitudinal argyrophil fibers are liberated, forming a delicate framework identical in every respect with the neo-endoneurium of a bundle of regeneration, whether innervated or not. This framework may be occupied secondarily by branching cells of endoneurial type; but the framework is not made by them any more than the framework of the bundle of regeneration is made by the endoneurial cells. It is the cylinder alone that determines the production of the framework.

In brief, the evolution of the elementary cylinder of a neurinoma is not only similar to but identical with that of the Schwann cell when, deprived of its neurite, it becomes an aneuritic bundle of regeneration. The sole difference consists in this, that in the neurinomatous cylinder the partitioning may be pushed further, dividing the cytoplasmic cylinder into a greater number of secondary cylinders, especially at a distance from the nuclei. In this event, each cylinder is extremely thin (barely 1 micron) but it is still enclosed in its argyrophobe sheath of collagen containing argyrophil fibrils.

CONSTITUTION OF THE BUNDLES

Having become familiar with the structure and evolution of an elementary cylinder, it is easy to understand how the bundles are constructed (Diagram 1). If the nuclei, the product of amitotic division, separate immediately from one another and range themselves along the length of the cylinder, the result will be a simple cylinder growing longer and longer. Between the widely separated nuclei, the longitudinal and incomplete partitioning of the cytoplasm and collagenization of the septa will convert the primitive cylinder into a linear, retiform syncytium similar to that which is produced in three months at the expense of and in the place of the

may separate, shift their position and range themselves in longitudinal rows (Fig. 4). Thus there is amitotic multiplication of the nuclei (in the many neurinomas studied, I have seen only one mitosis) with production of isogenic groups. When the group of nuclei remains in the same transverse plane, the cytoplasm of the cylinder broadens at this point; when the nuclei range themselves longitudinally, the cylinder elongates but remains narrow.

In cross-sections it is seen that the blue collagenous sheath still fits the cylinder closely, but it presents internal spur-like projections, simple at first, then branching, finally uniting with one another in a more or less continuous network. Here and there are points where blue septa appear in the heart of the cytoplasm with no apparent relation to those of the periphery, but soon making connections with them. Longitudinal sections show that these spurs are cross-sections of longitudinal septa and that the resulting network corresponds in reality to a cross-section of the longitudinal collagenous septa which cuts the cytoplasm of each primitive cylinder into smaller cylinders. They are tubular septa like those which enclose the primitive cylinder; but they are incomplete and spare the transverse connections of the initial cytoplasm.

The partitioning is always much more advanced at a distance from the nuclei than beside them. It is always followed sooner or later by cleavage of the collagenous septa and consequent separation into secondary cylinders. When the cleavage is precocious the cylinders are small; when it is late the primitive cylinders may acquire an enormous diameter before splitting (Fig. 3). It is conceivable that the predominance of small or of gigantic cylinders in such or such a neurinoma may give to these tumors quite different aspects without there being any essential difference in their structure.

(B) *Van Gieson*: This stain colors the spurs pale pink, the longitudinal fibers bright red.

(C) *Silver-Ponceau-Light Green*: This technique shows that the collagenous spurs and septa replace the cytoplasmic septa exactly (Fig. 3). Usually the longitudinal argyrophil fibers make their appearance in the amorphous collagenous septa (I have never succeeded in impregnating a web of circular fibers); less often they appear in the still cytoplasmic septa of the cylinders (Fig. 3 (b)). It is obvious that between the simple cylinder and the complex

degenerated medullated nerve fiber in the incised segment of a nerve in which regeneration has been prevented (see Part I).

However, as we have already noted, even in the experimental aneuritic bundle, there is very pronounced broadening in the region where its evolution is furthest advanced (the hernia). This broadening is always characterized by the presence of several nuclei in the same transverse plane. Conversely the active elongation observed in the zone of invasion is always characterized by shifting of the isogenic nuclei along the fiber. The nuclei are never placed one behind the other in a straight line parallel with the axis of the cylinder. This is due to the fact that in an isogenic group each nucleus occupies a different sector of the cylinder. When a nucleus, together with its cytoplasm, shifts along the lengthening cylinder, it remains for a time in the prolongation of the sector in which it first appeared; hence the irregular line of the shifted nuclei seen in longitudinal section.

In the bundles of the neurinoma, the nuclei behave similarly. Sometimes they are ranged in series along a cylinder (Fig. 4); sometimes they remain in the same transverse plane or nearly so. Under these conditions, when the partitioning and the subsequent collagenous cleavage reach the region occupied by an isogenic group of nuclei that have remained transverse, each nucleus becomes separated from its neighbor; but here, as in the internuclear zone, the separation is never complete. Always there persist transverse and sheathed cytoplasmic anastomoses which maintain the transverse symplastic continuity of the nucleated enlargements here as everywhere else (Figs. 1, 2 and 3 (*d*)). Secondarily these transverse anastomoses become slanting, due to unequal shifting of the nucleated enlargements, a shifting induced by unequal elongation of the bundles. From this time forward the anastomoses tend to run in a longitudinal direction like the bundles themselves.

We may suppose that in each bundle the retiform structure of three dimensions perpetuates itself indefinitely, owing to the alternating but capricious interplay of amitosis, followed sometimes by rapid longitudinal shifting of the nuclei, sometimes by precocious partitioning of the isogenic groups of nuclei that have remained transverse. Precocious longitudinal shifting of the nuclei and of their cytoplasm lengthens the cylinders and the bundles; precocious par-

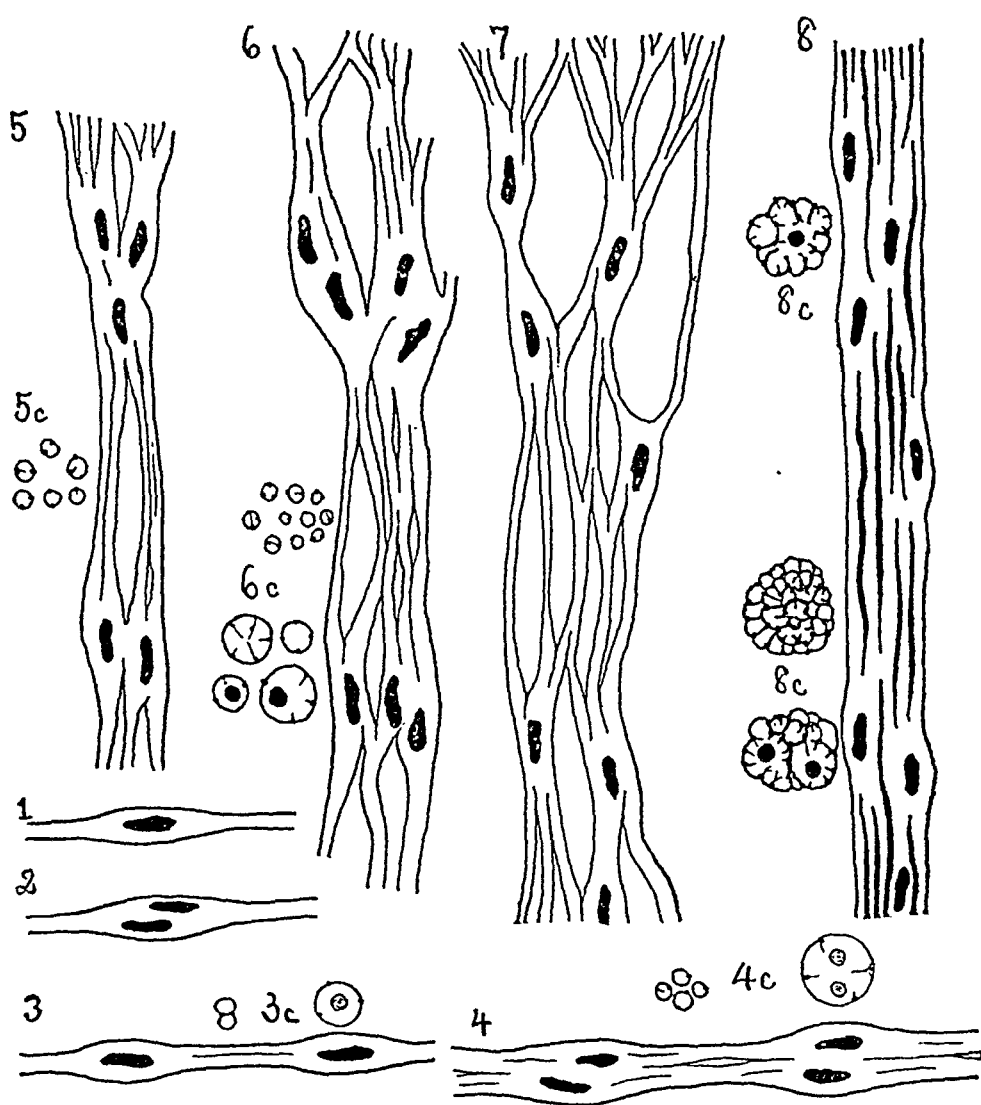


DIAGRAM I

Neurinoma. Evolution of a simple cylinder; its transformation into a bundle.

(4 to 7) The bundle in process of formation is dissociated by the edema.

(8) Aspect of bundle (7) not dissociated by edema.

(3c), (4c), (5c), (6c) and (8c) Represent cross-sections of the bundles bearing the same numbers.

tumor tissue before them and compress it. These palisaded nodules may be so voluminous or so numerous and confluent that they themselves constitute almost the entire tumor, the bundles being reduced to the position of occasional elements. The palisaded nodules differ from the bundles not only by their general aspect but also by their more rapid and independent growth.

Personal Observations: Francini showed long ago that in their structure the palisades do not differ essentially from the bundles. The techniques employed by me confirm this view.

RUDIMENTS OF PALISADES

On examining certain cylinders of bundles that are distinctly polarized but which have been dissociated by edema, there is often seen between two consecutive nuclei a transverse blue band that is striated longitudinally. This band consists of the many collagen sheaths that surround each of the tiny cytoplasmic cylinders resulting from very advanced cleavage of the primitive cylinder. Here the collagen is more abundant because there are more sheaths in close contiguity (Figs. 4 and 8). In bundles that are not so dissociated, several similar blue bands may be found on almost the same plane; by their juxtaposition they describe more or less straight palisades of collagen.

ELEMENTARY PALISADE SYSTEMS

(A) *Trichrome Stain:* Serial sections of a palisade system made at a right angle to the long axis of the nuclei and to the direction of the fibrils appear as follows:

In sections passing through a nuclear plane, the almost round nuclei are set close together in a common cytoplasmic mass; the cytoplasm is partially divided by incomplete collagenous septa. Many of the nuclei are fissured or they may form small groups (isogenic groups) enclosed in the same cytoplasmic body.

In sections passing through the ends of a nuclear plane (Fig. 9), a few nuclei are still seen but the collagenous septa separate them more completely than at the level just described. Alongside of the nucleated areas there are others free from nuclei, of the same caliber or smaller, in process of longitudinal cleavage.

tioning of the isogenic groups of nuclei multiples the cylinders and broadens the bundles.

In conclusion, from all points of view the elementary structure and the evolution of the fasciculated cylinders of neurinoma are similar to those of the schwannian bands in a nerve that has been sectioned but not innervated. In both situations the endoneurial cells are present during the changes in the bands and in the cylinders; but they themselves do not undergo any changes. The fundamental element of neurinoma therefore is the schwannian syncytium and not the endoneurial cell.

II. THE PALISADES

Classic Data: As observed in routine stains, the palisades consist of oval nuclei aligned in the same transverse plane "like staves of a barrel" (Verocay). On each side of this nuclear palisade, or on one side only, there is a fibrillar band parallel to it. The fibrils are oriented like the nuclei, running perpendicular to the nuclear band. With Van Gieson's solution they stain yellow or pink, sometimes red. With the silver method they impregnate like reticulin. When the sections happen to show the full length of the fibrils, it is seen that the fibrillar band is really stretched between two parallel nuclear palisades. It seems to unite them, forming a fibrillar palisade between two nuclear palisades.

Thus a "palisade system" is formed by two nuclear palisades parallel with each other and an intervening fibrillar palisade. Such simple palisade systems are frequent in neurinomas, but still more frequently the palisades present a more complex structure. Instead of being straight they may curve in more or less intricate ways. Several systems of palisades may be superposed to form an alternating series of nuclear and fibrillar bands parallel with one another; or the superposed nuclear palisades may appear in the sections as broken lines, sawteeth, anastomosing at their angles. In this way the nuclear bands describe a sort of net with lozenge-shaped meshes, the spaces being filled by the fibrillar palisades.

At some point these palisade systems are always attached to the bundles or to their reticulated, degenerated forms as seen in Type B. When complex, the palisade systems may attain considerable size, forming clearly circumscribed nodules which push the surrounding

In this description I trust that I have shown that in neurinomas the palisades have the same essential structural features as the polarized bundles. Therefore if the structural identity of the polarized bundle with the aneuritic schwannian bundle of regeneration leads us to consider the polarized bundles as of schwannian and not of connective tissue origin, we have every reason to believe that the palisade systems are schwannian also.

THE SCHWANNIAN PALISADES ARE PATHOGNOMONIC OF SCHWANNOMAS

Since the time of Francini, Verocay and Antoni, palisading has been accepted as pathognomonic of neurinomas. The partisans of the mesodermal hypothesis of these tumors oppose to this idea: (1) the palisade arrangement of the muscle fibers of the obliterated appendix first observed by Oberndorfer; (2) similar fibers in the stomach and intestine; (3) the palisades that are indisputably seen in certain leiomyomas; (4) and here I shall make every reservation,* in certain fibromas and spindle-cell sarcomas.

Let us say at once that the palisades which correspond either to a "state of repose" (appendix) or to a state of contraction (stomach and intestine) of the muscle fibers, or to "rhythmic disposition of the muscle cells" (Lauche, Krumbein), have none of the specific structural features of the schwannian palisades as I have just described them. There is lacking the ensheathing of the anastomotic prolongations stretched between two nucleated palisades by homogeneous collagenous sheaths containing argyrophil fibrils. It is conceded that neurinomas are not the only tumors possessing palisades; but to be pathognomonic of neurinoma the elements of the palisade must have the specific structure of the schwannian elements. Nestmann has expressed the same idea in another form.

SIGNIFICANCE OF THE PALISADES IN SCHWANNOMAS

We must now put to ourselves the question — what is the significance of these schwannian palisades? There is one consideration which dominates the problem first of all: certain neurinomas have no palisades. Nevertheless, in all of them the polarized bundles have the same structure.

* The palisaded "connective tissue tumors" invoked as arguments against the schwannian hypothesis are precisely these encapsulated tumors of the nerves.

In sections passing through a fibrillar plane, if the palisade is compact it appears to be formed of many pink dots in an undivided blue mass. Obviously each pink dot corresponds to the cross-section of a slender protoplasmic prolongation from the nucleated areas. Among them are found oblique sections also.

Following the series of sections, the same aspects appear in reverse order. The pink dots, representing the cytoplasmic prolongations, diminish in number, grow larger; then the nuclei embedded in their incompletely partitioned cytoplasm reappear.

When edema infiltrates a fibrillary palisade, it isolates each cellular prolongation from its neighbors and shows not only that each prolongation is provided with its own tubular sheath but also that each sheath is split off, separated from its neighbors and individualized. Between these collagenous tubes there are a variable number of collagen fibers running in the same direction (Fig. 10).

Thus each nuclear palisade is connected with its neighbor by a series of protoplasmic prolongations sheathed with collagen. Most of them connect the nucleated territory of one palisade with another exactly opposite to it in another palisade. Others run obliquely, uniting two adjoining prolongations or two nuclei not situated opposite each other. Obviously in the heart of the palisade system, there is a repetition of the retiform arrangement of three dimensions that we have described in the bundles. Examination of longitudinal sections confirms this view precisely (Figs. 5 and 6).

(B) *Laidlaw-Ponceau-Acid Fuchsin-Light Green Stain*: This technique reveals here too the presence of fibrils either embedded in the septa and sheaths or free in their interstices (Fig. 7). Scanty in the incomplete septa of the nucleated palisades, they expand abruptly in a sort of brush at the margin of the nucleated palisade; here they are distributed among the sheaths which enclose each cellular prolongation, from three to five fibrils to each sheath. These fibrils come together again close to the next nucleated palisade, but their point of reassembling is seldom opposite their point of departure (Figs. 7 and 19).

Thus the palisades offer three colors: pink dots (cross-sections) or pink lines (longitudinal sections) connecting the cytoplasm of two nucleated palisades, argyrophil dots or spindles, and in the interstices of these linear structures a diffuse green, corresponding to the ensemble of collagenous sheaths which surround each cytoplasmic prolongation.

The production of palisades has been explained as a sort of accident (Francini). Instead of dividing transversely and aligning themselves behind one another along the length of the fiber, the nuclei are assumed to divide longitudinally and take their position in a plane perpendicular to the axis of the original cylinder. Thus, it is said, the nucleated palisade is constituted.

Fortuitous Palisades: In this conception there is both truth and error. There are palisades which are produced by this simple mechanism. I shall call them rudimentary or fortuitous palisades. In my opinion, the nuclei always divide longitudinally. If secondarily they move lengthwise along the cylinder, the cylinder elongates. If the dividing nuclei remain in the same transverse plane, the cylinder broadens without elongating. A simple palisade system is formed when two such nucleated planes arise near each other along the same cylinder. The cylinder does not elongate, but the longitudinal cleavage persisting in the middle of the internuclear cytoplasm and the subsequent appearance of the collagenous sheaths, completes a simple neurinomatous palisade.

In these rudimentary forms the nucleated and collagenous palisades have the general form of the cylinders. In fact, they are segments of cylinders comprising two nucleated palisaded discs separated by a disc consisting of their prolongations ensheathed in amorphous and fibrillar collagen. These fortuitous palisades usually remain small; the cylinder containing them is scarcely, or not at all broadened.

The palisades produced by this simple and fortuitous mechanism are to be found in almost all neurinomas.* I mention them especially in order to distinguish them clearly from the palisaded nodules which seem to me to be of much greater theoretical interest.

Palisaded Nodules: If all of the palisades in neurinomas were due to this simple and fortuitous mechanism, they too should have a cylindrical form. Whatever their dimensions, they should consist of alternate nucleated and collagenous discs, all the broader in proportion as their growth is prolonged. However, this discoid structure, real as it appears to be in compact and voluminous palisaded nodules, is an illusion soon dissipated by their examination in serial sections, especially when an edema occurs to dissociate the various

* I speak here solely of the compact bundles unmodified by degeneration of their stroma (see Section III. Myxoid degeneration).

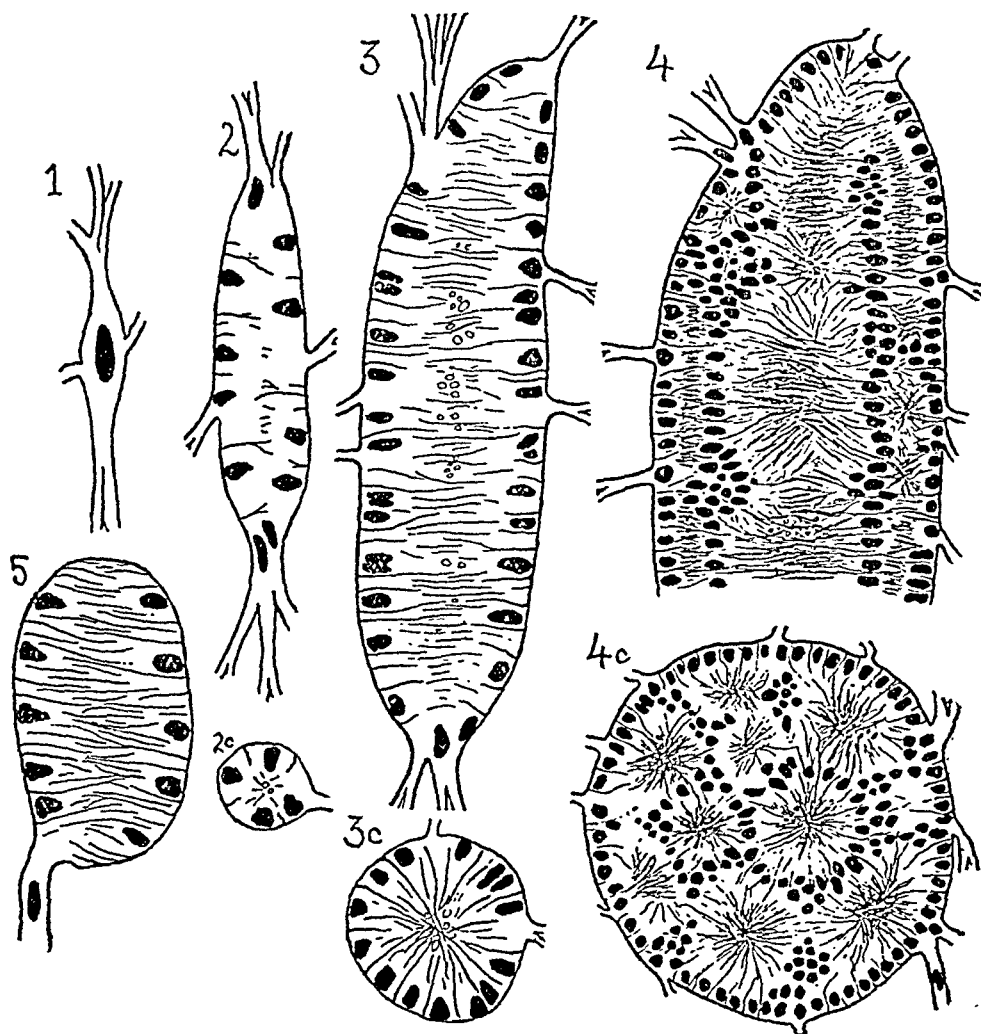


DIAGRAM 2

Neurinoma. Histogenesis of a palisaded nodule.

(1) Simple cylinder.

(2) Elongated and broadened cylinder; its nuclei marginal. Transverse septa are appearing (*cf.* Fig. 13).

(3) Continuation of the broadening, elongation and transverse partitioning. In this stage the meissnerian structure is typical (*cf.* Figs. 14, 15, 16). Between the marginal nuclei the striation of the collagenous palisades becomes accentuated.

(2c), (3c) and (4c) represent cross-sections of (2), (3) and (4).

(4) Appearance of new nucleated and collagenous palisades and production of a complex palisaded nodule (*cf.* Figs. 17 and 18).

(5) Supporting (schwannian) apparatus of a normal Wagner-Meissner corpuscle.

the cylinder thus transformed constitutes a simple palisade system; but at the same time it has exactly the structure of an aneuritic Wagner-Meissner tactile corpuscle, such as I have described in pigmented moles, but a corpuscle that is prodigiously long and usually curved (Figs. 14, 15, 16).

There is one structural characteristic that distinguishes these aneuritic tactile corpuscles from normal ones. Having arisen from a member of a retiform system and being continuous with their fellows both longitudinally and crosswise, the corpuscles have preserved their initial anastomoses with neighboring cylinders, both at their ends and along their sides.* Like the tactile corpuscles of pigmented moles, they are situated in the course of the retiform cylinders, not at the end of simple nerve fibers like normal tactile corpuscles.

Figures 14, 15 and 16, taken from a neurinoma of the shoulder, which I owe to the kindness of Professor J. McFarland of Philadelphia, show how typical these Wagner-Meissner corpuscles may be. The tactile corpuscles rarely remain in this typical stage of their evolution. As a rule they increase in bulk and grow more complex. The marginal nuclei become superposed transversely (Fig. 17). Between them there appear new anastomotic prolongations ensheathed in collagen. In this way the complex palisaded nodule is constructed (Fig. 18 and Diagram 2, No. 4).

DISCUSSION OF SECTION II

From this point of view the palisaded nodules lose the accidental character conferred on them in the classic conceptions. They become organoid formations resulting from a special evolution of the fasciculated cylinders. It is well known that this evolution is not presented by all neurinomas. Elsewhere I shall revert to the frequency and the inconstancy of the differentiation of tactile corpuscles in tumors of the peripheral nerves, both encapsulated and diffuse. I have already called attention to the almost constant presence of these tactile corpuscles in pigmented moles and I have demonstrated thereby that these tumors spring from the tactile nerves of the derma. The tactile corpuscles of pigmented moles are derived from Schwann cells; they are seldom innervated.

* The anastomoses multiply by the customary process, longitudinal cleavage, at the same time that the palisaded nodule itself increases in bulk (Diagram 2).

elements. The illusion is dissipated also by study of the histogenesis of the palisaded nodules.

On examining a palisaded nodule that has been dissociated by edema, it will be seen that each collagenous palisade does not really have the form of a disc included between two nucleated discs, but rather that of a long, sinuous cylinder, more or less flattened and branched, the branches anastomosing laterally with one another. Between these cylinders and applied to their surface there are branching cells, anastomosing, sheathed in blue, filling the interstices. The palisaded arrangement of the nuclei has disappeared.

The cylinders themselves are striated transversely by argyrophil fibers and by the ensheathed anastomotic prolongations of the cells which surround them. This cylinder and the cells which surround it form a structure exactly like the supporting apparatus of Wagner-Meissner tactile corpuscles, as their histogenesis shows.

PALISADED MEISSNERIAN NODULES

In my edematous schwannoma of the palate, certain fortunate sections furnished images like those of Fig. 13. Locally a cylinder has elongated greatly and at the same time broadened. For simple mechanical reasons it has become curved. Its nuclei, instead of elongating lengthwise of the fiber, have elongated crosswise; they have aligned themselves along the sheath but their axes are perpendicular to it. The partitioning, at first cytoplasmic, secondarily collagenous, instead of forming longitudinally has formed crosswise, incomplete here as always, leaving the continuity of the syncytium intact.

The nuclei multiply, remaining marginal. The cylinder broadens and now the collagenous septa multiply also. They are no longer longitudinal, but transverse in relation to the cylinder. They divide the nucleated territories incompletely, ensheathing the prolongations which connect these nucleated territories from one side of the cylinder to the other. Here, as in the bundles, collagenization is more precocious around the cytoplasmic anastomoses than around the nuclei. Consequently at this stage the axis of the cylinder is surrounded by a series of marginal nuclei, but the axis itself is occupied by multiple anastomoses ensheathed in collagen uniting the nuclei transversely or, better, diametrically.

At this stage, when examined in a longitudinal and axial section,

(a) In growing, the bundles and the palisades become richer in collagen. Not only do the collagenous sheaths and fibrils multiply as new cylinders arise from the old ones, but also the cleavage which converts a cylinder into a bundle liberates a certain number of fibrils which were formerly included in the collagen sheath (homologue of the Plenck-Laidlaw sheath). These fibrils, having become interstitial, are the homologues of those which constitute the interstitial endoneurium of the bands of aneuritic regeneration. As described in Part I, this endoneurium is determined by the Schwann cells and not by the endoneurial cells which, when they exist, limit themselves to occupying the endoneurium secondarily.

(b) In areas where the tumor cylinders and the palisades cease to grow, some of the cells degenerate and disappear. The products of degeneration are often incorporated by macrophages which become filled with lipoid vacuoles like xanthoma cells and usually gather around the vessels. The origin of these macrophages is undetermined; they may be either endoneurial cells become phagocytic or histiocytes.

When the tumor cells disappear, the collagen fibrils which formerly surrounded them remain; they come closer together and slowly lose their argyrophilia as their colorability by picro-fuchsin and aniline blue increases. There seems to be no increase in their number but simply a change in their tinctorial affinities. That which proves their schwannian origin is the fact that for a long time the architecture of the bundles and of the palisades can be recognized when every cell has disappeared from their interstices (Figs. 19 and 20). The collagen fibrils come together, not because they have multiplied, but because the schwannian bands which formerly separated them no longer exist. In the interstices of this collagen framework, become more dense by the disappearance of the Schwann cells, there is often no cell whatever, endoneurial or any other kind.

MYXOID METAMORPHOSIS

This state of degeneration is the one described by Antoni as Type B, the reticular type. It may be only partial, or it may affect the entire tumor. In extreme examples the neurinoma becomes cystic.

As for the genesis of Type B, I can only confirm Antoni's explanation, jellification and swelling of the collagenous fibrils and sheaths

What is logical for pigmented moles, is it not logical also for the encapsulated tumors of the nerves? May we not assume that those neurinomas in which the cylinders give rise to palisaded Meissnerian nodules have different organogenic properties from those neurinomas which do not produce palisades? In other words, is it not the tactile nerves that produce palisaded neurinomas? And are not non-palisaded neurinomas the product of non-tactile nerves, possibly motor nerves?

I know well that embryology, which has demonstrated the common origin of the entire schwannian system from the neural crest, does not yet authorize such an hypothesis; but the schwannomas in their two grand varieties confront us and demand an explanation. In default of two kinds of peripheral neuroglia, may we not suppose that the sensory and the motor neurites respectively, when they invade the initial cells of schwannomas before the beginning of their autonomous proliferation, in some way impregnate these cells, conferring on them and on their tumor-forming descendants specific organogenic properties? This is but another hypothesis, the experimental proof of which seems *a priori* unobtainable because laboratory animals, apart from the higher primates, possess no Wagner-Meissner corpuscles.

THE VESSELS

The vessels of neurinomas are capillaries. The vessel wall consists of endothelium resting on a thin collagenous sheath continuous with the collagen of the tumor. Often there are a few histiocytes in contact with this sheath. The intervals between the vessels are occupied by the compact tumor tissue already described in which the mesodermal (?) element is represented by the only cells of endoneurial type. I may remind the reader that these cells have not produced the collagen fibrils of the tumor; the collagen fibrils have been determined by the schwannian syncytium.

III. DEGENERATED FORMS

SCLEROSIS OF SCHWANNOMAS

This much discussed sclerosis, which partisans of the schwannian hypothesis believe to represent connective tissue invasion of the schwannian tissue, has in reality quite other significance. It is the product of two successive and opposing processes, growth and decay.

which I have given in the beginning of this paper. However, almost always the diagnosis of schwannoma may be made from the presence of typical cylinders which remain here and there, particularly around the vessels.

THE FIBRILS THAT STAIN BLUE IN PHOSPHOTUNGSTIC ACID HEMATOXYLIN

In neurinomas, Mallory's phosphotungstic acid hematoxylin often stains some fibrils blue, usually very few. The American school offers this as an argument for the connective tissue origin of neurinoma. For them, these are fibroglia fibrils and as such they are assumed to be characteristic products of fibroblasts. However, it should not be forgotten that the very name given by Mallory to these fibrils, which are seen in connective tissue and especially in young connective tissue, is derived from their staining with this reagent exactly like the fibrils of central neuroglia. Nageotte has shown that the gigantic bands of experimental schwannomas contain fibrils colorable by Benda's method for neuroglia. For my part, in the same material I have seen fibrils, doubtless the same, stain blue with phosphotungstic acid hematoxylin. These fibrils belong to the Schwann cells and not to fibroblasts. At any rate, as far as this staining reaction goes, the fibrils found in neurinomas that stain blue with phosphotungstic acid hematoxylin might be neuroglia fibrils just as well as fibroglia. This reaction cannot serve as an argument either for or against the mesodermal or ectodermal origin of a tumor.

SUMMARY AND CONCLUSIONS

The aim of this paper is to demonstrate that the fundamental element of encapsulated neurinomas is structurally identical with the syncytium of Schwann when that syncytium is undergoing autonomous proliferation, a proliferation that has been released by the disappearance of the neurites which normally inhabit it.

The schwannian elements of a neurinoma proliferate within the endoneurium, bounded by the old perineurium, now become the capsule, and not in the extraneural connective tissue. This proliferation within the endoneurium explains why the bundles and their palisades are as free from individual lamellar sheaths (perineuria) as are the aneuritic bundles resulting from transformation of de-

of the cylinders and of the palisades. The sheaths disappear first, the fibrils next, commencing with the finest ones (Figs. 21, 24, 25); the collagenous palisades are affected last. The effect of this jellification is to separate the tumor cells without breaking their anastomoses; from this point of view it has the same effect as the 1 per cent solution of nitric acid used by Nageotte in the study of degenerated nerves. As in the acid, the jellification swells the endoneurial collagen and reveals the continuity of the schwannian syncytium in three dimensions. It has another and unexpected result. Deprived of their sheaths, which have become jellified, denuded and accompanied by a few collagen fibrils, the Schwann cells resemble and are mistaken for connective tissue cells, or for endoneurial cells. Nevertheless there can be no possible doubt of their origin: study of the bundles or of the palisades in process of jellification shows that it is really the cells of the cylinders and of the palisades that undergo this metamorphosis.

This myxoid transformation is always accompanied by profound changes in the blood vessels. The most constant lesion is hyalinization of the connective tissue coat proper of each capillary, a hyalinization which surrounds the endothelium with a sheath that may be very thick and may constrict or obliterate the lumen of the vessel (Fig. 25). To this hyalinization is often added local multiplication of the capillaries, forming here and there tiny glomeruli with hyaline walls very like those observed in many central gliomas.

It is probable that the myxoid transformation of neurinomas results from unfavorable circulatory conditions connected with this hyalinization of the vessels. It begins at a distance from the vessels, the tumor tissue in contact with the vessel still preserving its compact structure; but it ends by involving the tissue around the capillaries. It may be followed by atrophy and disappearance of the cells, in place of which there remains nothing but a fluid holding in suspension some fibrillar débris. The usual corollary to this cellular disappearance is the presence of lipoid macrophages around the vessels.

In certain instances this jellification of the sheaths is not accompanied by cellular atrophy; proliferation continues. It is obvious that it is not in tumors of this kind or in the areas presenting Antoni's Type B that one should seek to verify the description of the schwannian cylinders enclosed in their collagenous sheaths,

vitalistic sophism based on artifacts. However, if this hypothesis should ever have any basis in fact, it should be found in regenerating nerves and in schwannomas; for what is the membrane of Schwann, the delicate cuticle which borders the schwannian cytoplasm and which collagenizes secondarily, if not an exoplasm which has at least the merit of existing?

In reality, the collagenous framework of the bundles of regeneration and of schwannomas is not secreted, neither is it produced, either by mesodermal cells or by Schwann cells. It is determined by the Schwann cells just as basement membranes are determined by epithelium. In the light of these facts, it is probable that the classic conceptions of the mesodermal origin of the endoneurium should be revised.

I have once more to thank my friend, Dr. George F. Laidlaw, for the scrupulous accuracy of his translation and for his tireless industry in presenting my ideas to the English-reading medical public.

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generated fibers in the incised segment of a nerve (Experiment I) and in nerve grafts (Experiment II).

The palisaded nodules are no developmental accidents. They are organoid productions. Their histogenesis and structure are comparable to those of the schwannian supporting apparatus of the Wagner-Meissner tactile corpuscles.

Since the palisaded nodules are not found in all neurinomas, may we not ask if their presence or absence is not related to two different organogenic properties of the Schwann cells? Despite the single origin of the Schwann cells as they leave the neural crest, may there not evolve two peripheral neuroglia, a sensory and a motor neuroglia?

In the experimental schwannomas of the rabbit as well as in the spontaneous schwannomas of man, the collagenous framework of the schwannian bundles is determined by the schwannian syncytium and not by the endoneurial cells. The endoneurial cells occupy the framework secondarily.

In schwannomas the sole collagen of mesodermal origin is that of the vessel walls. All the rest of the collagen is determined by the neurectodermic syncytium.

The sclerosis of schwannomas does not result from their invasion by connective tissue but from condensation of the framework when deserted by the Schwann cells, and also at times by all cells.

For these many reasons, at the beginning of this paper I have insisted that neurinomas contain mesoderm only on the same terms as do central gliomas, no more and no less. I have no illusions of the fate in store for a doctrine so tainted with heresy. The universally held dogma that collagen is the direct and specific product of connective tissue cells, mesodermal by definition, will long confront me. Nevertheless this dogma is indefensible.

No cell, not even the fibroblast, produces collagen. As demonstrated by Nageotte, the fibroblast determines the coagulation of collagen in its vicinity but does not secrete it. Doubtless the cell operates by means of a ferment which coagulates an albuminoid of the interstitial substance to form collagen, just as a thrombocyte acts on the fibrinogen of the blood plasma by a ferment which coagulates it in the form of fibrin.

As far as it concerns the collagen of connective tissue, the exoplasm hypothesis maintained by Studnicka and by Laguesse is a

green and black collagen appears in the heart of the schwannian syncytium. At no point in the preparation or in serial sections of the same bundle is there a single endoneurial cell to be found. All of this collagen therefore is determined by the syncytium of Schwann alone.

On the right and below are three small schwannian cylinders of the same preparation highly magnified ($\times 1800$). Partitioning is more advanced in the non-nucleated region. On the right there is substitution of collagen (green) for the red cytoplasmic septa. The argyrophil fibrils are embedded in the green collagen sheath.

FIG. 4. Schwannoma of the palate. Edematous region. Trichrome stain.

Schwannian cylinders growing longitudinally (*cf.* Experimental Schwannomas, Part I, Fig. 5). Retiform arrangement. The cylinders show distinct striation, especially between the nucleated regions, all the more pronounced since each red striation (protoplasmic prolongation) is ensheathed in blue (*a*) (rudiments of palisades). The nuclei are dividing by amitosis into isogenic groups (*b*), which break up rapidly as the nuclei range themselves in rows along the fiber. Around the cylinders in the edematous tissue there are several inflammatory cells.

FIG. 5. Schwannoma of the palate. Edematous region. Trichrome stain.

Palisade system. The nuclei are arranged in three planes, (pl 1), (pl 2), and (pl 3), (nucleated palisades). The cytoplasmic bodies of each plane are connected with those of neighboring planes by slender anastomotic prolongations ensheathed in collagen. They are retiform, all elongated in the same direction and grouped in small bundles. The ensemble of these collagenous sheaths (see their real structure in Fig. 6) constitutes the classic fibrillar and collagenous palisades which separate the nucleated palisades.

(*a*) Mononuclear cytoplasmic body.

(*b*) Amitotic division of a nucleus and formation of an isogenic group.

(*b'*) Isogenic group; the uppermost cell begins to separate from the others.

(*c*) Ensheathed anastomotic prolongations.

(*c'*) Cross-section of bundles of cytoplasmic prolongations uniting two isogenic groups belonging to plane 1 (pl 1) but situated below and above the section. These groups of nuclei, now separated, had a common origin in the same primitive isogenic group.

(*b''*) A cell of group (*b'*) shifts longitudinally and parts from its fellows in order to occupy another plane, where it may become the point of departure of another nucleated palisade situated between (pl 1) and (pl 2).

FIG. 6. Schwannoma of the palate. Non-edematous region. Trichrome stain.

Palisaded nodule growing both longitudinally and transversely. On both sides of the center of the figure, palisaded systems superposed and cut in various planes; (*l*) longitudinal; (*t*) transverse; (*o*) oblique. In each nucleated palisade the nuclei are ranged not only crosswise (staves of a barrel) but also lengthwise; this indicates lengthening of the cell which contains them. The lengthening of the cell makes preparation for the appearance of new collagenous palisades in the present nucleated palisades.

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DESCRIPTION OF PLATES

PLATE 67

FIGS. 1 and 2. Schwannoma of the palate. Edematous region. Trichrome stain.

Isolated bundle of schwannian cylinders, most of them cut across. All of them are enclosed in a continuous collagen sheath (blue) intimately adherent to their cytoplasm. Their caliber varies greatly. The broadest cylinders present either one nucleus (*a*) or several nuclei in an isogenic group (*b*). Almost all of the cylinders present collagenous septa, either rudimentary in the form of spurs projecting inward from the sheath or in the form of lines in the middle of the cylinder. This partitioning is finished early around the cellular prolongations (*c*); it proceeds more slowly around the isogenic nuclear groups (*b*). Partitioning is followed by cleavage of the collagenous septa (*e*). The cleavage converts the primitive cylinder into a bundle (*d*). (*f*) Endoneurial cells. (*g*) Simple cylinders.

FIG. 3. Schwannoma of the palate. Non-edematous region. Silver impregnation of the reticulin (Laidlaw) followed by staining with the ponceau-acid fuchsin mixture, phosphomolybdic acid and acetic light green.

In this very thin section (2 microns) the cytoplasm seems to be vacuolated. In reality they are almost colorless. The red lines cutting the cylinders are the cytoplasmic septa which little by little turn into collagen; in this way the solid cylinder is cut up into a bundle as in Figs. 1 and 2. The sheaths and the collagenous septa instead of being blue seem to be formed of two substances, the one amorphous, colored green, the other fibrillar and argyrophil. The argyrophil fibrils seem to be embedded in the green substance.

The letters (*a*), (*b*), (*c*), (*d*) and (*e*) have the same significance as in Figs. 1 and 2. At (*g*) note two giant bundles with groups of isogenic nuclei. The bundles are in process of partitioning but not yet split apart. The



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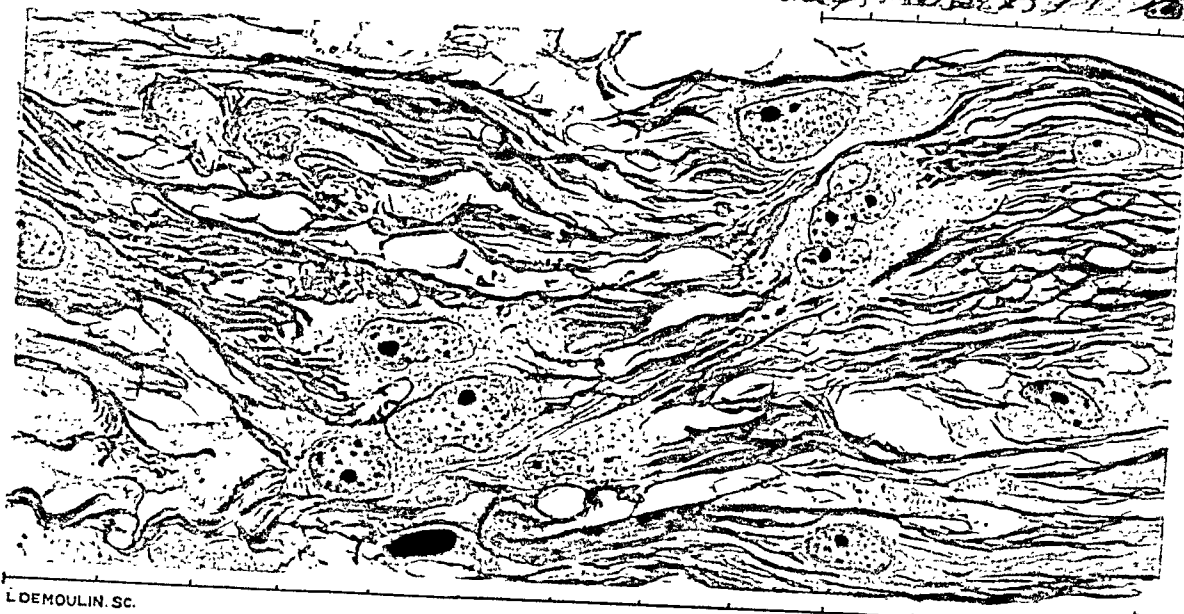
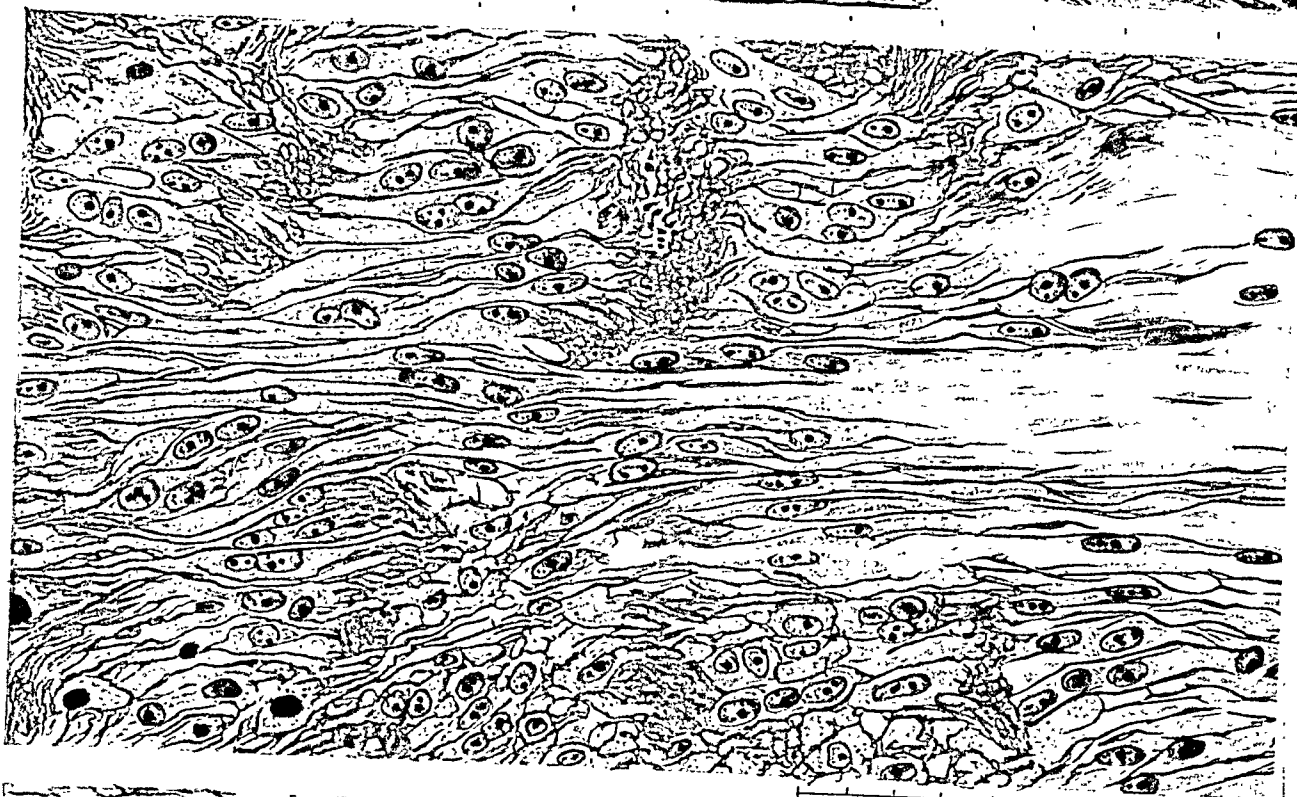


FIG. 8. Schwannoma of the palate; very edematous region. Trichrome stain.

On the left a cross-section of a group of bundles. The nucleated territories are separated incompletely by collagenous septa. The partitioning is more complete in the bundles that are cut across between the groups of nuclei (rudiments of palisades). On the right a group of bundles cut a little obliquely. In the center of the group, young cylinders are completely split and separated from one another. Below bundles in process of elongation cut lengthwise. In the interstitial edema are several isolated cells without sheaths; these are endoneurial cells.

FIG. 9. Schwannoma of the palate; slightly edematous region. Trichrome stain.

Slightly oblique section of a complex palisaded group in lozenge form. Above, the section passes through the nuclei, which are incompletely separated by collagenous partitions. Upper center, the border of a collagenous palisade similar to the one in the center.

In the center is a "collagenous" palisade constituted in reality by collagenous tubes containing cellular prolongations. In the center of the figure the tubes are cut across; they connect two nuclear planes, one above and the other below the level of the section. In the lower part they are oblique, connecting nucleated planes above and below the level of the section.

FIG. 10. Schwannoma of the palate; very edematous region. Trichrome stain. $\times 1500$.

Cross-section of a sclerosed palisade. The cellular prolongations ensheathed in collagen are recognized as tiny black circles. Some of them are free; most of them are united in small bundles. The collagen fibrils appear as lines or dots. On the left these fibrils seem to be embedded in the tubular sheaths of the cellular prolongations.

FIG. 11. Schwannoma of the palate; non-edematous region. Laidlaw silver-ponceau-light green.

Longitudinal section of a bundle. The cylinder in the center presents a slight shrinkage on each side of which the nuclei are spaced lengthwise. At the point of shrinkage the collagenous sheath is visible in front view. Note the web of criss-cross argyrophil fibrils which constitutes it. In the meshes a gray tint (green in the section) represents the pellicular collagenous membrane in which the fibrillar net is embedded.

In the center of the figure the elongation exists almost alone and constitutes a typical straight bundle in the heart of the palisaded nodule. Just as the palisades take their origin from the bundles, so the bundles may spring from the palisades.

FIG. 7. Schwannoma of the cervical region. Beginning of atrophy. Laidlaw silver-ponceau-acid fuchsin-light green.

Palisade of irregular type, lozenge-shaped, cut perpendicularly to the general direction of the plane of the palisades. The line of section is that of line h-p on the tracing of Fig. 6.

(a) Palisaded nuclei embedded in cytoplasm. The cytoplasmic territories are separated by incomplete collagenous septa which respect the transverse anastomoses. A certain number of cells have disappeared, leaving their collagenous niches vacant (a').

(b) "Collagen fibers" directly connecting two nucleated palisades situated above and below the section. These fibers have been cut across. Each fiber is complex and contains an axial red dot, the cross-section of a minute protoplasmic cylinder, ensheathed in green. In the green sheath are seen black dots which correspond to cross-sections of reticulin fibrils. These so-called "collagen fibers" then are really minute prolongations of the cells belonging to and connecting the two superposed nucleated palisades, each prolongation being sheathed both with amorphous collagen and with reticulin.

(c) Other oblique and branching prolongations establish lateral anastomoses between nucleated palisades and between their prolongations.

(d) These black spots correspond to bundles of collagen fibers; their brush-like ramifications furnish the argyrophil fibrils visible at (a) (see Fig. 19).

PLATE 69

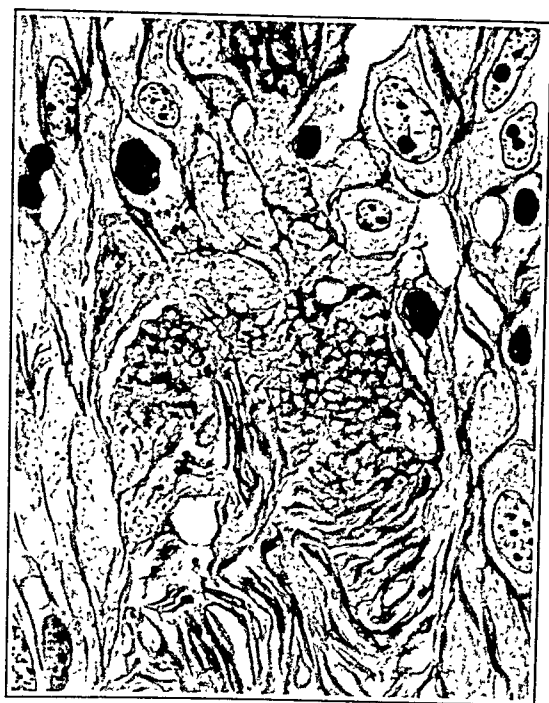
FIG. 12. Pulp of index finger. Normal Wagner-Meissner corpuscle.

FIG. 13. Schwannoma of the palate. Beginning of the evolution of palisades. Long, curved and broadened cylinder. The nuclei are marginal and oriented perpendicularly to the sheath. Between them appear collagenous septa, no longer longitudinal but transverse.

FIGS. 14 and 15. Typical meissnerian evolution.



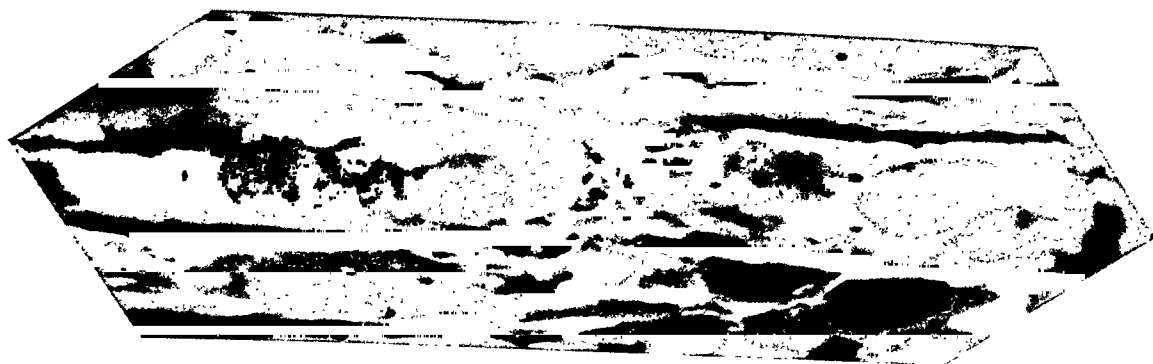
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Masson

Experimental and Spontaneous Schwannomas. II

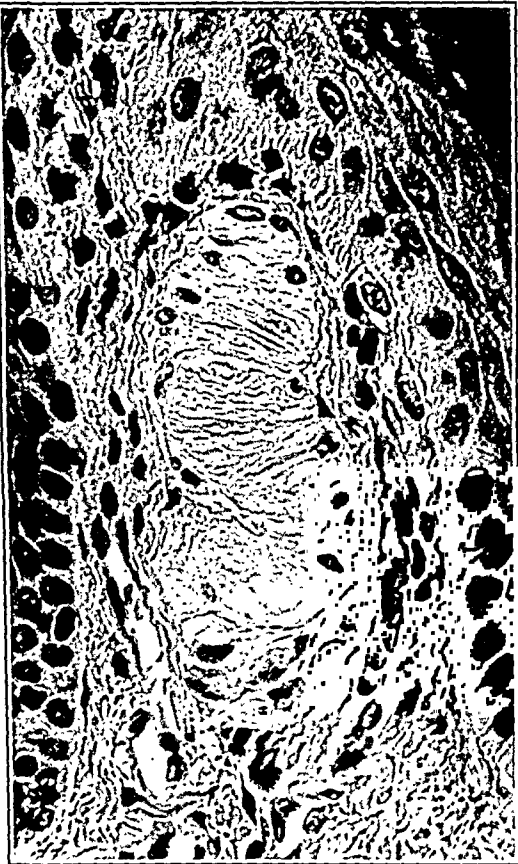
PLATE 70

FIG. 16. Broadened meissnerian corpuscle. The collagenous palisading becomes more pronounced.

FIG. 17. Intermediate form between a meissnerian corpuscle and a typical palisaded nodule. Beginning of the formation of a secondary palisade.

(FIGS. 14, 15 and 17 were taken from the same preparation of a neurinoma of the shoulder which I owe to the kindness of Professor McFarland, of Philadelphia.)

FIG. 18. Schwannoma of the palate. Complex palisaded nodule.



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15

Masson

Experimental and Spontaneous Schwannomas. II

PLATE 71

FIG. 19. Schwannoma of the palate. Laidlaw silver-ponceau-acid fuchsin-light green.

Sclerosis of a palisade. Rarefaction of the tumor cells. Persistence of the collagen. The gray tint corresponds to collagen sheaths almost entirely deserted. The figure shows particularly clearly the arrangement of the argyrophil collagen in the palisaded systems.

FIG. 20. Schwannoma of the cervical region. Trichrome stain.

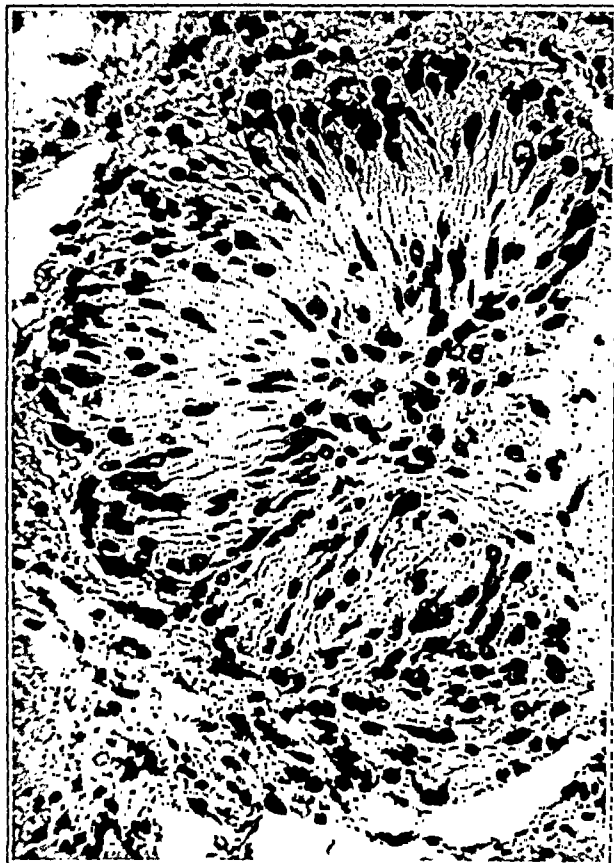
Total sclerosis; slightly edematous region. There are no more cells but only the collagenous framework of the neurinoma. Here all argyrophilia has disappeared. Nevertheless in the upper part of the figure we recognize the characteristic arrangement of the palisaded systems and below the arrangement of the bundles.

FIG. 21. Schwannoma of the forearm. Trichrome stain.

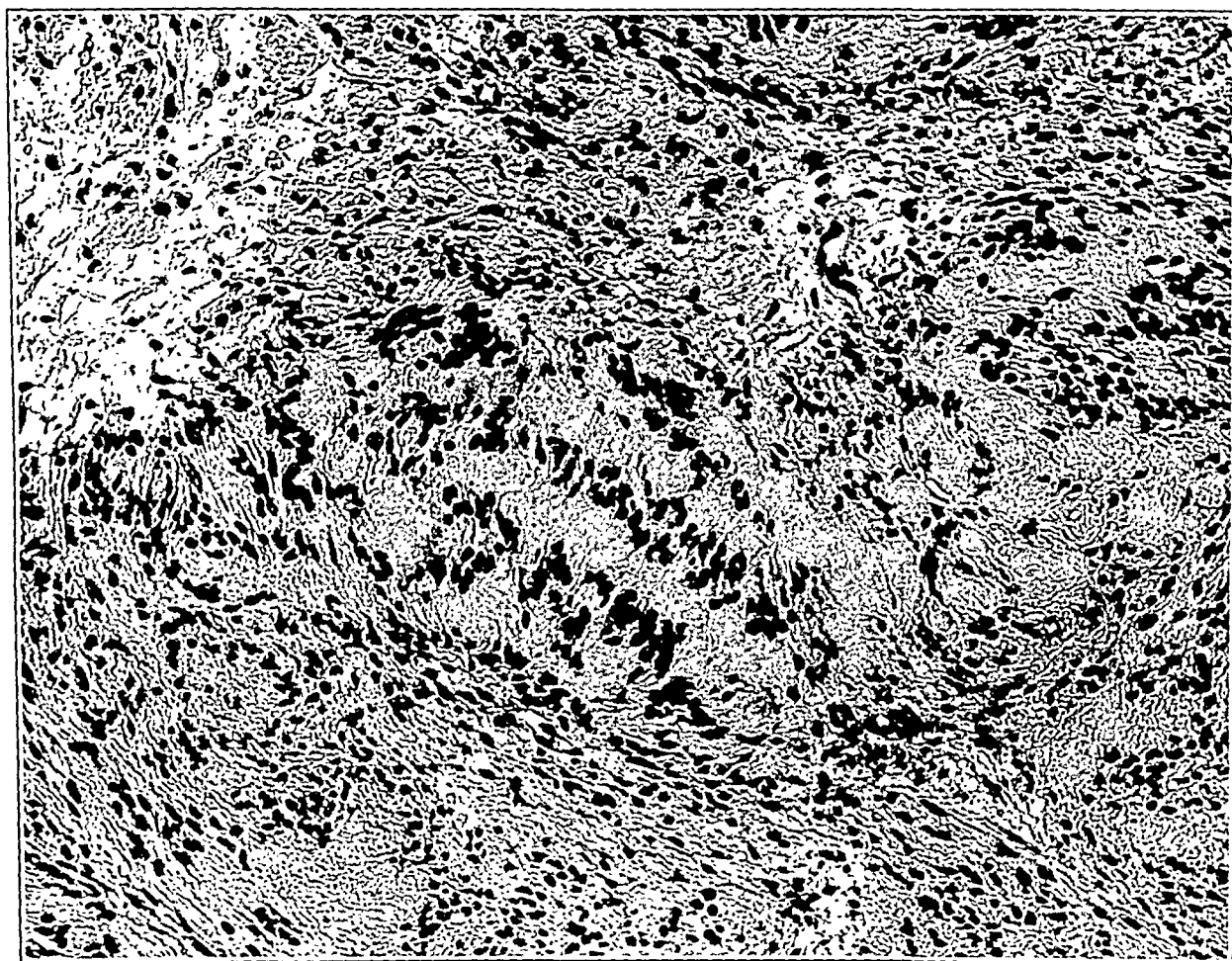
Beginning jellification of the collagen in a bundle. Beginning of Antoni's Type B. The sheaths and the interstitial fibers have become indistinct. The cylinders are separated by an amorphous substance stained feebly by the aniline blue.



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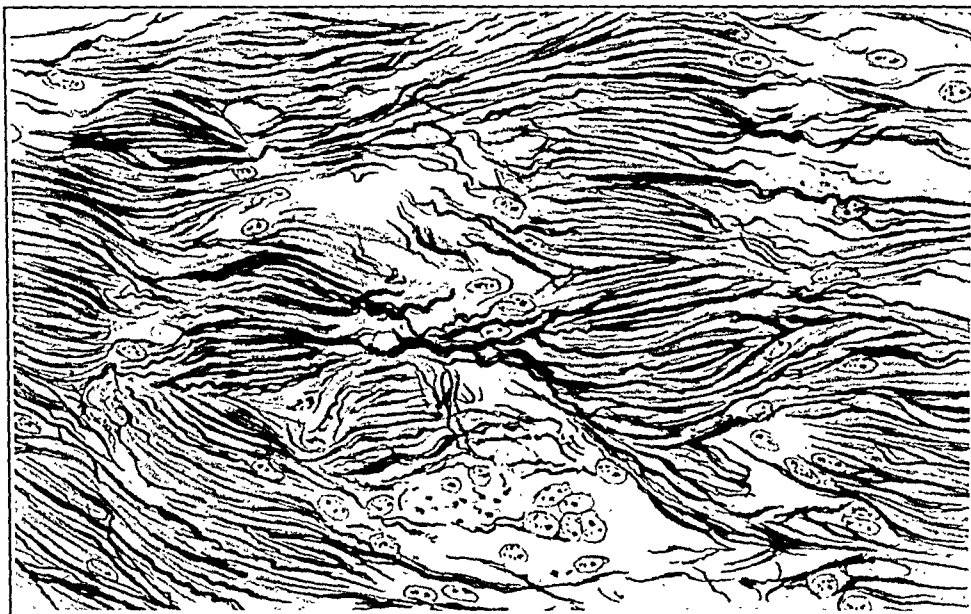
PLATE 72

FIG. 22. Schwannoma of the forearm. Trichrome stain.

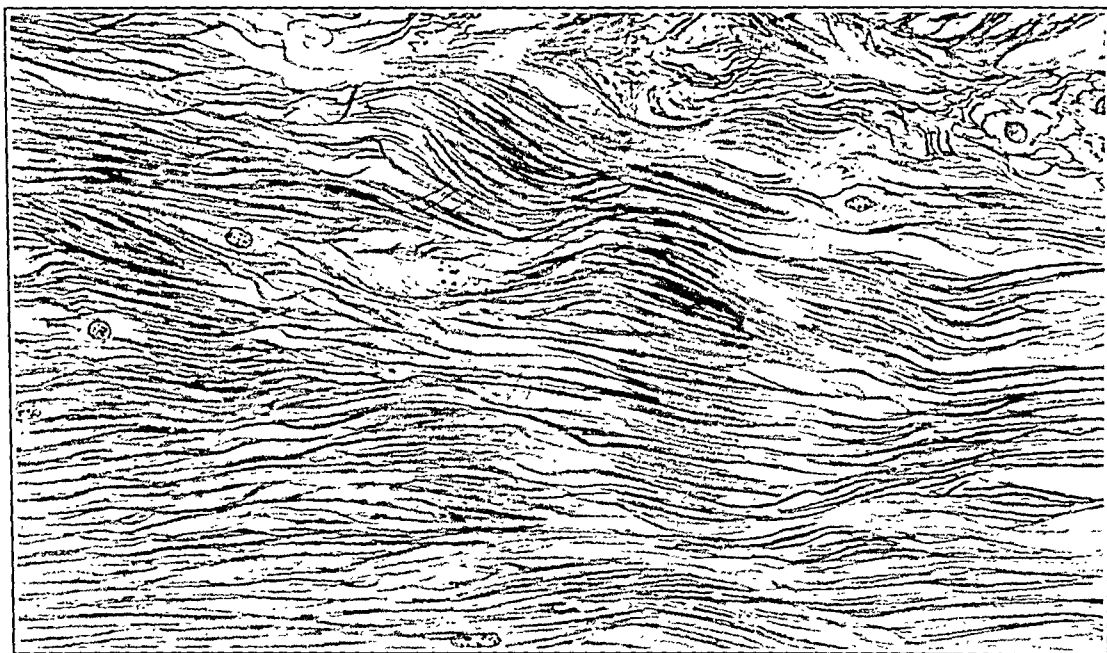
The same bundle. The region represented lies to the right of that seen in Fig. 21. The cells, while remaining anastomosed and fasciculated, are separated by the amorphous bluish substance. They no longer have a sheath. A few bluish, swollen fibrils are recognizable with difficulty. This is a perfect example of Antoni's Type B.

FIG. 23. The same tumor. Trichrome stain.

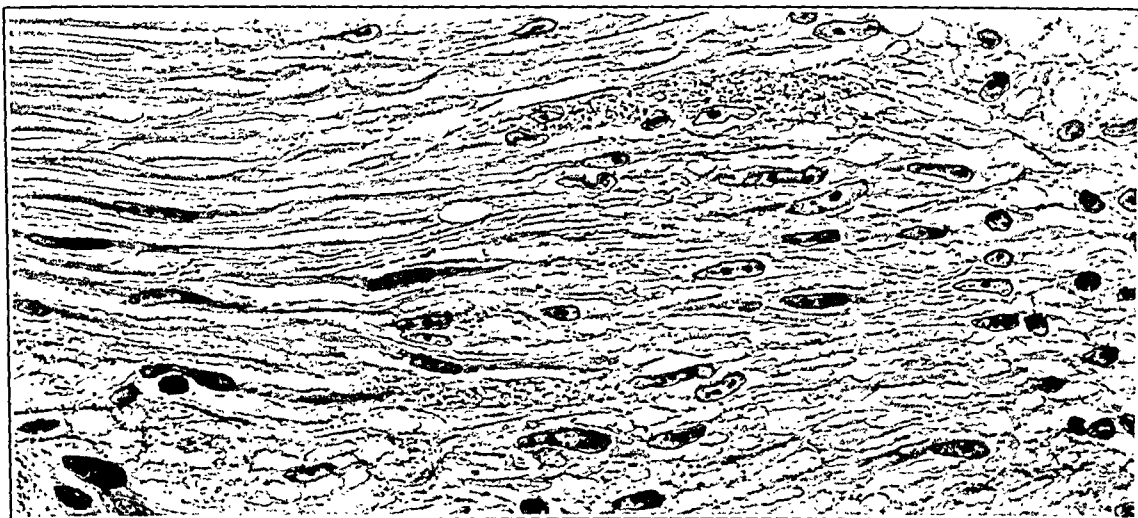
Cross-section of a jellified bundle. The figure shows the anastomoses which connect the prolongations of the same cell with one another and also the anastomoses of the cell with its neighbors. At this stage the Schwann cells are distinguished with difficulty from endoneurial cells. The three-dimensional retiform structure of the schwannian syncytium is particularly clear. The silhouettes of the cells represented in these two figures recall strangely those of oligodendroglia.



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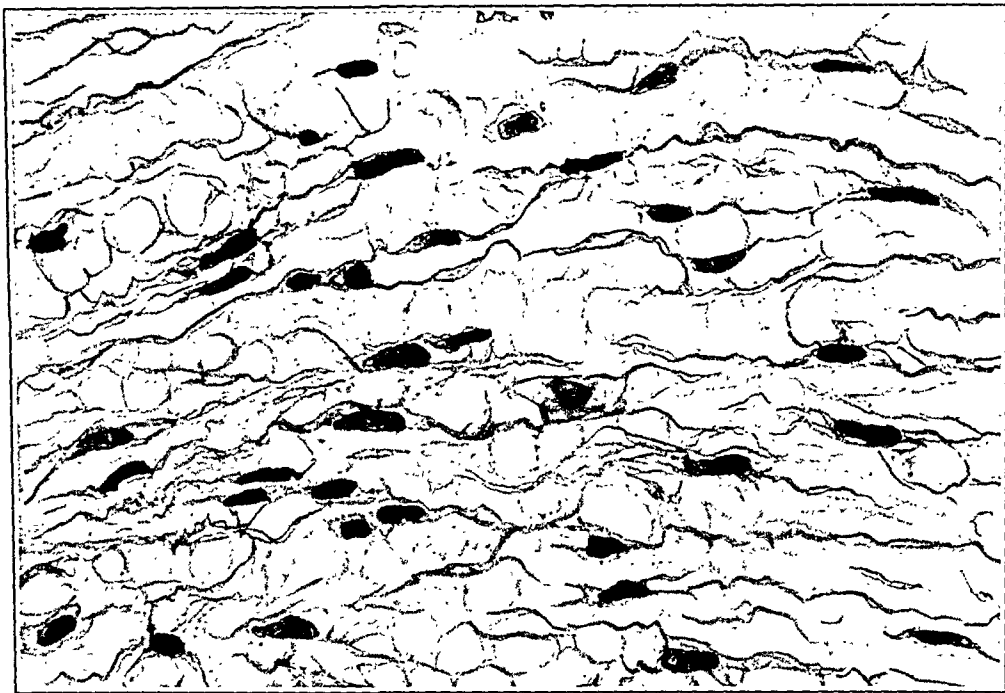
PLATE 73

FIG. 24. Schwannoma of the forearm. Laidlaw silver-ponceau-acid fuchsin-light green.

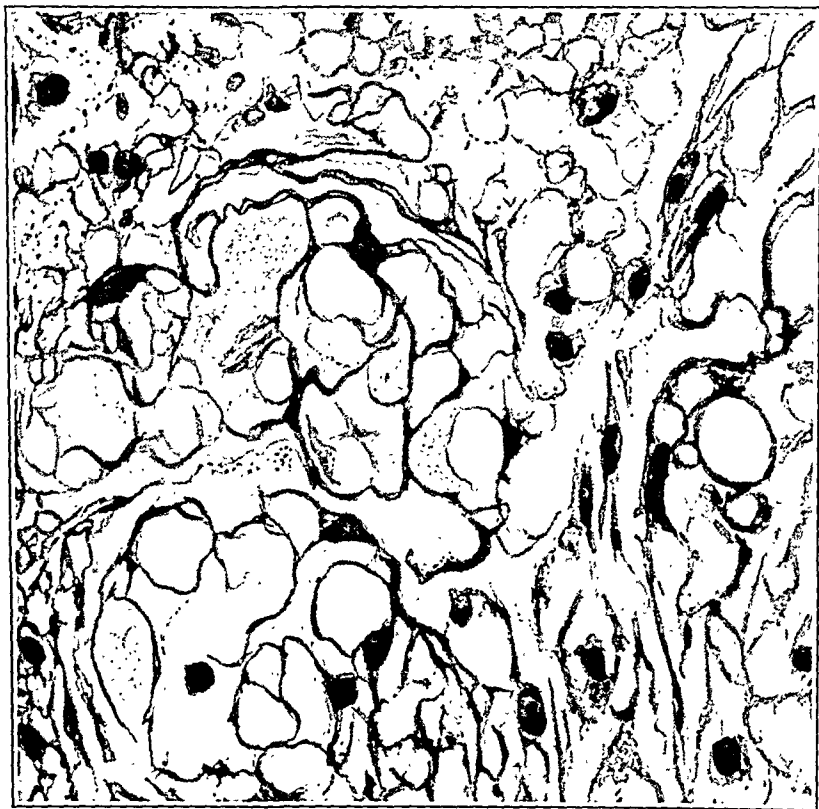
On the left a cross-section of a compact bundle. The sheaths are a little thickened. Cross-sections of argyrophil fibrils are seen clearly as dots. On the right a jellified bundle. The cylinders no longer have sheaths. The argyrophil fibrils have become scanty. Cavities mark the places of the autolyzed cells.

FIG. 25. Schwannoma of the forearm.

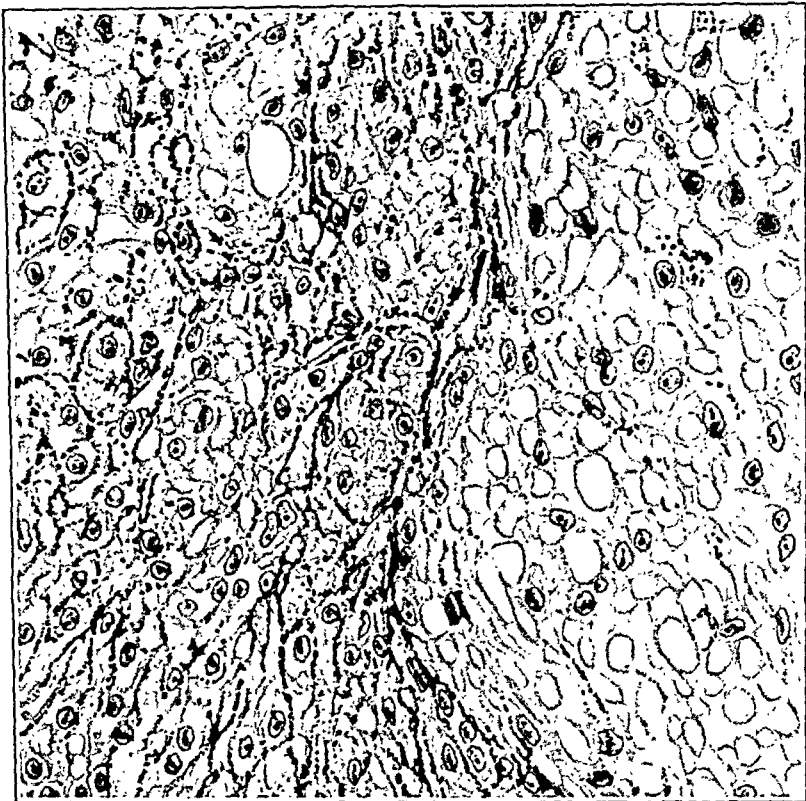
Antoni's Type B. Hyalinized vessel forming glomerular groups. In the cartouche, one of these groups more highly magnified. The lumen of a knotted capillary has been cut across at four different points. This vascular arrangement is very frequent in certain central gliomas.



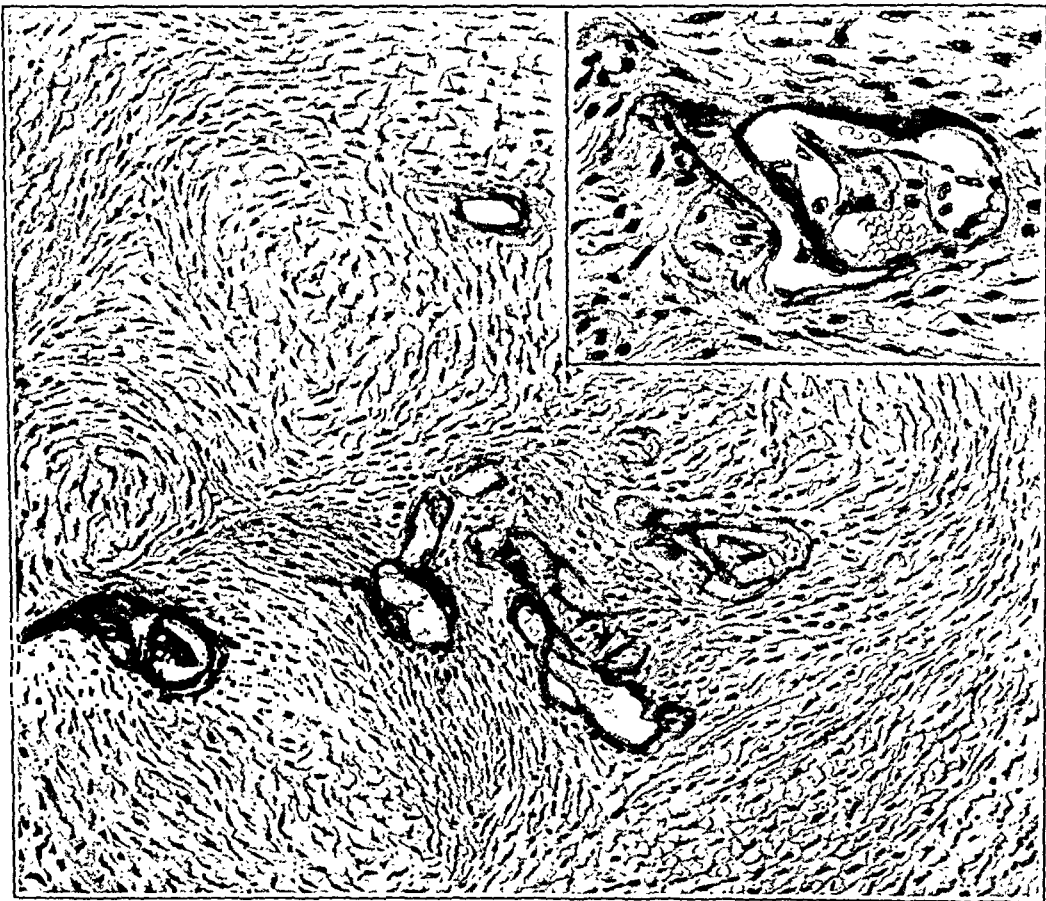
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in further detail, giving some of the evidence for the view stated, and the disabilities of the implantation were discussed.

In the present contribution it is proposed to elaborate the thesis by consideration of the mechanism of the transformation of several forms of follicles into epithelium-lined blood cysts.

The primitive form of the graafian follicle — the primordial follicle — consists of the ovum with one layer of cells of the stratum granulosum. As the follicle becomes mature, these cells proliferate until they are several layers thick. Later a cavity (the antrum folliculi) appears and the ovum, surrounded by cells of the stratum granulosum, projects into this (Fig. 1). At this stage the cells of the stroma immediately surrounding the follicle swell and become spheroidal, constituting a theca interna, and around this the stroma cells arrange themselves in a layer which is described as the theca externa.

The changes that occur normally and which may result in the formation of the cysts to be considered are those of proliferation and evolution on the one hand, or retrogression on the other. The former process gives rise to the corpus luteum and can only take place in a completely mature follicle which has ruptured, with projection of the ovum into the peritoneal cavity. The retrogressive changes which result in the formation of the atretic follicle in its various forms occur in the great majority of follicles.

A reference to many current books and papers indicates that the significance of these bodies is not generally appreciated. Atretic follicles are confused with graafian follicles, the hyaline and fibrous bodies arising from them — the corpus candicans, corpus fibrosum, and so on — are intermixed with the corpus albicans, and the occasional bodies in which the cells come to resemble luteal cells are most often confounded with the corpus luteum. It will be apparent that, in the absence of a knowledge of these structures, the accurate interpretation of pathological conditions occurring in the ovary is impossible.

In my experience, the multiple blood cysts may show morphological characteristics which correspond to any of the bodies occurring normally in the ovary. Also, although relatively few main paths are followed in the development of the blood cysts, at the same time very many by-ways may be taken in the production of what are finally similar results. The life history of the maturing graafian follicle may

THE ORIGIN OF EPITHELIUM-LINED BLOOD CYSTS (CHOCOLATE CYSTS) OF THE OVARY FROM THE GRAAFIAN FOLLICLE AND ITS DERIVATIVES*

E. S. J. KING, M.D., M.S., F.R.C.S., F.R.A.C.S.

(From the University of Melbourne, Melbourne, Australia)

The epithelium-lined blood cysts of the ovary have been discussed extensively during the last three decades under various terms, of which the most important are adenoma endometrioides ovarii,¹ perforating chocolate cysts,² ectopic Müllerianoma,³ adenomyoma of the ovary,⁴ endometriosis,⁵ endometrioma and endometriomyoma.⁶ The majority of these terms tacitly assume that the material comprising the cysts is aberrant endometrium. Pick's term "endometrioides" emphasises the similarity of the tissue to the endometrium of the uterus. "Adenomyoma" is a noncommittal term and the suggestion of the "perforating chocolate cysts" is unsatisfactory in view of the ambiguity (from the pathological point of view) surrounding the term "perforating." I would submit, for reasons to be given, that the term "multiple chocolate (or tarry) cysts" is sufficiently descriptive and does not wed us to any hypothesis, which in the light of new observations may have to be abandoned.

A number of hypotheses have arisen to explain these cysts, of which the most important are those of Iwanof and Sampson. The former postulated a metaplasia and downgrowth of the surface epithelium of the ovary, and in other regions, of the peritoneum. Sampson suggested an actual transplantation of endometrium from the uterus to the ovary and other organs.

In 1929 the writer⁷ brought forward evidence showing that many of these cysts arose from the bodies normally occurring in the ovary — namely, the graafian follicles and their derivatives. At first, proof of this hypothesis could not be obtained in all cases, and in 1930 the similarity of tarry cysts to examples of endometriosis (the nature of which could not be demonstrated) was discussed.⁸ Later, further evidence was obtained in the case of the previously unproved examples. In another paper⁹ the subject was considered

* Received for publication July 30, 1931.

ture of one of these is accompanied by the retrogression of the others. This has been shown experimentally, in birds by Pearl and Surface¹⁰ and in rodents by Herrmann and Stein,¹¹ to be due to the presence of a hormone produced by the developing corpus luteum. Since the number of times that ovulation in any ovary occurs is limited (300 to 500) and there are many thousand ova in the organ, the reason for the tremendous excess of bodies derived from atretic follicles over those arising from true corpora lutea is apparent. These bodies are, therefore, much more important — at least numerically — than the corpora lutea.

Various forms of retrogression are encountered:

1. Absorption.
2. Atresia with cystic development.
3. Atresia with proliferation of the stratum granulosum and cyst formation.
4. Atresia with proliferation of the stratum granulosum without cyst formation.*

1. *Absorption*: The commonest mode of retrogression of the graafian follicle takes place *in situ* with the least amount of disturbance of the surrounding tissues. The ovum dies, the stratum granulosum proliferates and, in lower animals, forms projecting masses beneath the zona pellucida. The granulosa cells invade the zona pellucida and the ovum. The membrana limitans becomes thicker and the cells of the theca interna proliferate. These gradually hyalinise, giving rise to a fibrous body possessing a hyaline capsule. This is termed the corpus atreticum. Characteristically it is composed of a central zone of material resembling connective tissue and a peripheral zone of hyaline tissue. The collapse of this body, which usually occurs, results in the corpus candicans. Occasionally some disturbance of the normal mechanism results in the production of a morphological variant — all or most of the tissue being fibrous with little or no hyaline material. Such a body is designated a corpus fibrosum.

Sometimes small spaces arise in one or another part of these structures. These may occur in either fibrous or hyaline bodies. It has

* One form of atresia observed in lower animals, described as rupture atresia,¹³ in my experience does not appear to have a constant correlative in the human ovary. The rupture of many of the atretic follicles containing blood, which is so characteristic of the multiple tarry cysts, is a possible counterpart.

be divided into two stages: (1) before an antrum folliculi is formed, and (2) after the formation of the follicular cavity. These will be considered separately.

THE PRIMITIVE GRAAFIAN FOLLICLE

The examination of the retrogressive changes in the primitive follicles of the human ovary is difficult, but in the case of ovaries of animals in which the ova are large (fish, reptiles, birds and, of the mammals, the Monotremata) the changes may be followed with ease. Subsequent examination of higher mammalian and particularly human follicles is thereby facilitated.

The ovum disintegrates and this appears to be the primary morphological change. The cytoplasm stains more deeply, becomes vacuolated and the nucleus becomes first pyknotic and then disintegrates. The cells of the stratum granulosum invade the ovular material, remove it by phagocytosis and finally the cells hyalinise, giving rise to a small hyaline body known as the corpus restiforme.

The pathological variant of this process is the formation of a small cyst, owing probably to some modification in the mechanism of absorption of the ovular material. Usually the cyst remains of a comparatively small size.

If the stratum granulosum has become three or four layers thick, some of these cells disintegrate and disappear so that a single layer is to be found. In many cases there is little evidence, except for the nature of the surrounding structures, for the determination of the origin of the small cyst, but in other cases there are remnants of a theca interna which make its nature certain. Though they are usually spherical, partial collapse of these cysts gives rise to various appearances in sections. When these structures occur in the ovaries containing "endometrial" cysts, they contain blood and are lined by a single layer of cuboidal, and rarely columnar, cells which often possess cilia. It is of interest that some of the earlier accounts of "graafian follicles" contain a reference to ciliated cells lining the cavity.

THE MATURING FOLLICLE

The vast majority of the graafian follicles present in the ovary do not become mature, but undergo atresia. Just before the time of ovulation a number of follicles are approaching maturity. The rup-

and collapse of the cyst results in a crenation of the wall. A characteristic appearance is obtained on section.

Atresia is frequently associated with haemorrhage, particularly in the region of the theca interna (Fig. 5), but in some cases, especially in the ovaries which are the site of the epithelium-lined blood cysts, bleeding occurs into the cavity of the cyst (Fig. 4). It is stated in the literature that blood does not find its way into the cavity of the atretic follicle, but an examination of the typical atretic follicles in the ovaries mentioned above will reveal some which show this peculiarity.

When blood finds its way into the cavity of these cysts, an epithelial * lining develops. Apparently the presence of blood is necessary for the development of this lining. This epithelium appears to arise from the few remaining cells of the disintegrating stratum granulosum. The reasons for this conclusion are: (a) the epithelium occurs in the cysts at the same site as the stratum granulosum, *i. e.* just internal to the theca interna; (b) rarely both may be found in the same cyst; and (c) the cells are epithelial in nature, *e. g.* they possess cilia.

Examination of the walls of a series of cysts thus shows all gradations between atretic follicles with a well developed stratum granulosum and theca interna without blood, and those which contain blood and show some degenerative changes in the wall on the one hand, and the hyaline and fibrous cysts either with or without an epithelial lining on the other. At first the stratum granulosum is about five layers thick and the theca interna is composed of well formed spheroidal cells. Then the stratum diminishes in size though the theca remains markedly in evidence. Finally the stratum becomes one layer thick, though still recognisable as such. At some stage blood appears in the cavity. Epithelial cells now appear at the site of the stratum granulosum and seem to arise from it. They may be syncytial at first, but for a time, even after cell boundaries are obvious, the cells are very irregular in form (Fig. 6). Later the cells become definitely cuboidal or columnar. The origin and nature of such a cyst is beyond doubt because the theca interna is still well marked (Figs. 3 and 4). Lastly there are those cysts in which the

* It is to be appreciated that the term "epithelium" is used only in the most general sense. The ovarian structures are derived from the coelom and are thus mesothelial in origin. The argument that epithelium does not give rise to phagocytic cells (with which incidentally I disagree, but do not propose to discuss here) does not apply.

been suggested that the cells which line such a space are endothelial in origin. Occasionally, however, a number of the cells of the stratum granulosum remain and the lining cells may be seen to be continuous with these.

Well marked, epithelium-lined glands may be found in such relationship to masses of hyaline and fibrous material as to suggest that they arose from the spaces referred to, and indicate that they arose from the follicle which gave rise to the hyaline or fibrous material. The significance of such masses in juxtaposition to glands described as "endometrial" has apparently been overlooked or regarded as incidental by observers.

2. *Atresia with Cystic Development*: The formation of follicular cysts, varying from the size of a pin-head to one-half inch or more in diameter, is an everyday observation to investigators of ovarian histology and pathology. The frequency of their occurrence causes us to regard them as being a normal method of retrogression of the graafian follicle.

The changes which take place imitate those occurring without cyst formation. The ovum disintegrates and the cells of the stratum granulosum begin to disappear also. The cumulus disappears at an early stage, and thus is formed a cyst lined uniformly by a layer of granulosa cells three to five layers thick (Fig. 2). At the same time the theca interna proliferates and forms a well defined layer outside the stratum granulosum. With further retrogression the stratum granulosum becomes still more reduced until only one layer is to be observed. The theca interna remains in its well developed state. Hyaline change usually occurs in the theca interna and the membrana limitans may be very well developed. It is important to appreciate that both hyaline and fibrous changes, which were described in regard to the absorption of the follicle *in situ*, may occur here. Usually some of the theca interna remains as a recognisable structure, but when it is replaced completely by hyaline and fibrous tissue the recognition of the nature of the cyst may be difficult. A knowledge of these transformations and the discovery of all gradations between the follicle commencing to undergo atresia and the cyst with only hyaline material in the wall, render the identification of structures of doubtful origin practicable.

While these changes are progressing, alteration in the shape of the cyst takes place. Apparently absorption of the cyst contents occurs

These cysts have been described by a number of writers. An excellent account is given by Shaw.¹² He points out that the cells which, though atypical, resemble true luteal cells, may arise from both the stratum granulosum and the theca interna. The cysts containing derivatives of both layers he refers to as "tarry granulosa luteal cysts," and those in which all remnants of the stratum granulosum have disappeared as "tarry theca luteal cysts."

Typical examples of each of these varieties are easily diagnosed, but many intermediate cases occur. Probably some of the tarry theca luteal cysts arise directly by luteinisation of the cystic atretic follicles described in the preceding section, but others arise by complete disintegration of the granulosa representative of the tarry granulosa luteal cyst. Brakeman and Shaw have described the occurrence of a heterotopic epithelial lining in such cysts. They have referred, however, mainly to the large single and double cysts which are generally recognised as being luteal. I would emphasise here that similar, though smaller, cysts occur amongst the multiple, epithelium-lined blood cysts described as "endometriomatous."

The sporadic cystic corpora lutea atretica occur in two principal forms: (1) the cysts in which the tissue closely resembles luteal tissue; and (2) the cysts in which the cells are very atypical even at an early stage.

(1). In the first group the cysts are easily recognisable since the cells resemble typical luteal cells, but the marked festooning of the cyst wall, characteristic of the derivatives of the true corpus luteum, is absent. Degeneration of the cells and their replacement by fibrous and hyaline material results in atypical appearances, but all gradations are to be found and experience enables one to interpret even the most atypical forms. Haemorrhage may occur into these cysts at any stage and a heterotopic epithelial lining frequently develops. It is of importance that both the retrogressive changes and the development of the epithelium are relatively late results in the life history of the cyst, and thus the epithelium is to be found in most imposing form in those cysts in which there is least evidence of the nature of the cyst. This is in all probability the reason for the opinion that the multiple, epithelium-lined blood cysts are not of local ovarian origin.

The retrogressive changes occurring in the walls of these cysts do not occur uniformly and in some cases it is possible to find luteal

epithelium is indistinguishable from that of the "endometrial" cysts and there is an underlying fibrous cellular stroma. Often the general conformation of the cyst suggests a relationship with the atretic follicle even in absence of other evidence (compare Fig. 5 with Figs. 12 and 13).

The distinguishing feature of the first two methods of atresia is a relatively large development of the theca interna. In the next two groups the atretic processes are characterised by a disproportionate overgrowth of the stratum granulosum, so that the resulting structure may bear some relationship to a corpus luteum.

3. *Atresia with Proliferation of the Stratum Granulosum and Cyst Formation:* The most characteristic form in which overgrowth of the stratum granulosum takes place is in the multiple luteal cysts associated with hydatidiform mole. Atresia of a large number of follicles occurs and results in bodies, of greater or less size, sometimes solid but more frequently cystic which resemble, both in their general structure and in the nature of their cells, corpora lutea; hence the term — multiple "luteal" cysts. When it was appreciated that the process was one of atresia, the principal group of cells was thought to arise from the theca interna as in the other forms of atresia mentioned. The work of several investigators, however, showed that these cells arose by proliferation of the stratum granulosum. These "luteal" bodies undergo retrogressive changes similar to those seen in the atretic follicles of the normal ovary, and fibrous and hyaline bodies result.

Haemorrhage occurs into some of the cysts, the number of which varies in different specimens. As in the case of other blood cysts of the ovary, physicochemical changes gradually occur in these blood cysts, resulting in a change of the blood to a tarry or chocolate consistence.

The walls of these cysts are frequently lined by a definite epithelium (Fig. 8). In these structures particularly can the continuity of this epithelium with the luteal cells (*i. e.* the modified stratum granulosum) be demonstrated.

In addition to these multiple cysts, similar cysts may occur in some ovaries singly in a sporadic fashion. They may contain blood which gradually becomes tarry. Retrogressive changes take place in the wall, the "luteal" cells become fewer in number and atypical in appearance, and the tissue adopts a fibrous or hyaline character.

4. *Atresia with Proliferation of the Stratum Granulosum without Cyst Formation*: This results in the production of small bodies consisting for the most part of cells indistinguishable from the phagocytic cells described above. Degenerated cells, débris arising from the breakdown of these and blood cells, and at times considerable amounts of well formed red corpuscles occupy the centre of these structures. They seem to arise in non-cystic atretic bodies into which small haemorrhages occur at an early stage.

This form of atresia is described here as a separate variety and well formed bodies are characteristic, but all gradations are found between these and the cystic forms referred to above.

Small spaces arise in some of these bodies. The débris found in the centre of the structure contains cholesterol and related substances, and the phagocytic cells unite to form syncytial masses around the material. Distension of the space occurs until the peripheral cell mass is but one layer thick. On the wall of some of the larger spaces an epithelium definite and columnar is found (Fig. 9).

THE CORPUS LUTEUM

After rupture of the mature graafian follicle, the stratum granulosum commences to proliferate to an astounding degree, giving rise to the large mass of cells comprising the convoluted walls of the luteal body. The cells also enlarge until they become the characteristic luteal cells. The theca interna also proliferates and gives rise to an irregularly placed layer of "paraluteal" cells. The central cavity may contain a considerable amount of connective tissue, but a space containing fluid, even though small, is almost invariable. The spot at which the graafian follicle ruptured on the surface heals, but it can usually be recognised as a depression of the exterior of the organ and by the projection of the corpus luteum towards this site.

Four stages of development may be recognised.

1. *Stage of Proliferation or Hyperaemia*: The granulosa cells begin to luteinise, but at this stage the theca interna cells look much more like the adult luteal cells. The corpus luteum is, at this stage, an inconspicuous vesicle of greyish colour — usually flattened.

2. *Stage of Vascularisation*: The luteal layer becomes invaded by newly formed capillaries pushing in from the theca interna. Whether haemorrhage does or does not occur at this stage is a much debated

tissue in one part of the wall, hyaline tissue obviously of luteal origin in another, and material indeterminate in character in another. This circumstance and the discovery of gradations between the typical "luteal" cyst and the epithelium-lined blood cysts determines the follicular origin of the "endometrial" cysts beyond doubt. Hyaline tissue is frequently described in the walls of blood cysts by the protagonists of the implantation hypothesis.

(2). The atypical forms comprise a group in which the walls are lined by large cells with small nuclei and voluminous vacuolated protoplasm. The cysts undoubtedly arise from luteal bodies of the atretic series. The problem is the nature of the cells.

These cells are undoubtedly phagocytic in character. They arise in greatest numbers in those cysts in which haemorrhage has apparently occurred at an early stage. The question is whether they are histiocytes or derived from the luteal cells.

There is not sufficient evidence at present to enable one to dogmatise upon this point, but I am of the opinion that the cells are derived from the luteal cells (usually the derivatives of the stratum granulosum) for the following reasons:

- (a) The phagocytic activity of the cells of the stratum granulosum in some forms of atresia, in lower animals, is undoubted.¹³
- (b) The morphology of such cells is that of typical phagocytes (of histiocyte type). This is a morphological change dependent on a change of function.
- (c) Evidence of remnants of both layers — stratum granulosum and theca interna — is to be found and the cells of both layers are atypical and resemble phagocytes.
- (d) Where luteal cells and phagocytic cells are found together intermediate forms may be seen.
- (e) Gradations from typical follicles showing similar cells which are obviously of granulosa origin to the atypical varieties described here are found.

These cysts develop a heterotopic epithelial lining. This lining may be present only in one part of the cyst, it may completely cover the wall or it may be patchy in distribution. The epithelium * in some cases is continuous with the phagocytic cells (Fig. 10).

* See footnote on page 422.

time so that tarry material and fresh blood may be seen occasionally in the same cyst.

Corpus luteum cysts may be lined by the luteal cells, by a connective tissue layer internal to the luteal cells, or by a heterotopic epithelium lying internal to the connective tissue — as pointed out by Fraenkel.¹⁴

Since relatively so few graafian follicles mature and rupture on to the surface, only a few corpora lutea are formed, and therefore cysts arising from this structure are found much less commonly than those derived from the atretic follicle. Furthermore, the characteristic feature of ovaries, which are the site of “endometrial” glands and cysts, is the observation that all the bodies found in the organ are undergoing retrogression⁹ — immature and mature follicles of various forms are affected. It would appear that no follicles are allowed to come to maturity during the active stage of the disease; some factor, possibly excess anterior pituitary hormone or related substance, is causing atresia and haemorrhage in all the follicles.

However, in the ovaries which contain multiple blood cysts, an occasional cyst of definite corpus luteum origin is to be found. Such cysts are always ancient.

Cysts of the corpus luteum are usually large, but quite minute though perfectly formed derivatives are to be found in the ovaries mentioned. The relationship of such structures to a healed stigma reveals their nature even when other criteria of differentiation are absent.

RÉSUMÉ

A great many structures occur in the ovary derived from the graafian follicle, most of them by the process of atresia, some by way of evolution and the corpus luteum. It is not practicable at present to give a complete account of all possible forms, but it is intended to draw attention to some of them. So many changes are still without adequate explanation, and the amazing number of appearances are so intricate and difficult of elucidation that a finished statement is not attainable.

The principal feature is that cysts of very many varieties may possess an epithelial lining and some of them, without thorough study of the ovary, will not be referred to their correct relationship with the follicles.

question. It probably does take place in some cases, though most authorities deny its occurrence until the next stage.

3. *Stage of Maturity:* The zenith of this stage is reached just before menstruation commences. It is now the characteristic yellow body of text-book description. The well formed luteal cells are separated from the lumen by a more or less well marked layer of connective tissue. The theca interna is represented by the collections of cells described by Pinto as "paraluteal." Haemorrhage occurs into the cavity of the body.

4. *Stage of Regression Marking the Onset of Menstruation:* There is a shrinkage of the luteal layer and fatty degeneration of the cells occurs. It is the presence of the fat in the cells which is responsible for the yellow colour of the "typical" corpus luteum. The cells now atrophy — pyknosis and ultimately disintegration of the nuclei occur. Connective tissue cells advance from the theca zone into the luteal layer and gradually replace the cells. Hyaline tissue appears, but whether this occurs in the connective tissue or in the luteal cells is as yet undecided. (I would subscribe to the second view.)

In the final stage the replacement of the luteal tissue by hyaline material is complete. The body produced is known as the corpus albicans. This is characteristic, but the corpus candicans arising from the atretic follicle is frequently mistaken for it. The corpus albicans, however, is usually larger, is composed of larger convolutions of hyaline tissue and possesses evidence of a central cavity.

CYSTS OF THE CORPUS LUTEUM

Cysts of the corpus luteum, at an early stage, show typical luteal tissue in the wall. As the cyst becomes older this tissue becomes replaced by hyaline material and, as happens in the case of the atretic follicles, sometimes by fibrous tissue. These changes are to be found in the older cysts and the fibrous tissue is best developed in the cysts which have become distended.

Should the luteal tissue become uniformly and completely replaced by hyaline tissue a corpus albicans cyst is formed. Many variations depending on the type and extent of the change taking place in the luteal tissue may arise.

Any of these cysts may contain blood and, as the cysts age, changes occur in the blood resulting in the formation of chocolate or tarry material. Haemorrhage takes place into the cysts from time to

The epithelium may be found in three circumstances: (1) where the epithelium completely lines the interior of the cyst; (2) where only a portion of the cyst is so lined; and (3) where the epithelium lines part of the cyst and is in direct continuity with granulosa or luteal cells which are lining the wall elsewhere.

Subepithelial Stroma

Beneath the epithelium there develops a loose tissue containing small round cells, spindle cells, obvious phagocytic cells and some pigment. The comparison of this tissue with similar material lining non-epithelialised blood cysts and in which absorption of the cyst contents is occurring, suggests that this tissue is taking part in the absorption of the blood. Its degree of development is closely associated with the development of the columnar character of the epithelium.

This stroma is frequently likened to the stroma of the uterine mucosa. Actually this is more a gross than an intimate histological resemblance.

The first stage in its formation appears to be a swelling of the subepithelial tissue, extreme vascularity and accumulation of fluid — “oedema.” Some observations suggest that this may be dependent on some alteration of cyst tension (Fig. 12), though this idea is difficult of application in many cases. That alterations of tension are present is shown by the collapse of the cysts. The areas described frequently occur on the portion of the wall which projects into the cavity.

Later and sometimes without the intervention of the preceding stage, a number of small round cells accumulate and some of the connective tissue cells swell. Phagocytic cells containing a large amount of protoplasm with varying amount of blood pigment and possessing small nuclei appear.

Gland Formation

“Gland” formation may occur in two principal ways: (1) from small atretic follicles — each follicle becoming a gland space; and (2) as subsidiary formations from larger cysts.

Of the second variety, the simpler form is that due to distortion of cysts which have collapsed. The distortion of the wall results in the formation of crypts. These crypts, if cut obliquely in section,

This brings us to the question of terminology. Since they arise from the numerous bodies of the ovary a general term is desirable. The term luteal cyst is applied to several forms of the larger cysts. Follicular blood cyst is applied to a different condition and does not include the derivatives of the corpus luteum. The most characteristic macroscopic features are their multiplicity and their blood content. Therefore, until their origin and nature are fully understood and recognised, the term "multiple chocolate (or tarry) cysts" seems adequate.

The Epithelium

The epithelium lining these cysts may be flattened, cuboidal or columnar. Each type of epithelium may be found lining a cyst completely, or all varieties with gradations between them may be found in the same cyst. In early examples the epithelium is nearly always flattened, but in older cysts (the age being shown by the presence of hyaline material) the epithelium becomes columnar. The characteristics of the columnar epithelium, which suggest that it plays a part in an absorptive mechanism, will be dealt with in another paper.

The origin of the epithelium lining tarry luteal cysts has been a source of speculation for a considerable time. Three possible origins have been suggested, namely: (1) from the surface epithelium of the ovary growing in at the stigma of the ruptured graafian follicle; (2) from endothelium by metaplasia; and (3) from luteal cells or their precursors the cells of the stratum granulosum.

The first can apply only to corpus luteum cysts and seems undoubtedly to be the explanation in some cases, since it is possible to trace the epithelium of the cavity in continuity with the surface epithelium. The endothelial origin does not seem to be adequate when some of the characteristics of the epithelium — the tall columnar form and the presence of cilia — are considered. The characteristics suggest an origin from epithelial cells and the writer is satisfied that the evidence definitely establishes the luteal (or granulosa) origin of the heterotopic epithelium for the majority. Even in the cases in which the epithelium develops from the surface epithelium, the cells are probably comparable with those lining luteal bodies arising from atretic follicles, since the stratum granulosum and the luteal cells are genetically related to the surface epithelium.

epithelium without due regard to the other parts of the cysts. It has been assumed that because some of the cysts do not possess epithelium they have previously possessed such a lining, and by some process (*e. g.* pressure atrophy) have lost it. The opposite view, that these cysts are an earlier stage in which the epithelium has not yet formed, should be seriously considered.

The labour expended by many investigators on the multiple blood cysts has established them as a clinical entity and has attracted attention to their special features. The conception of aberrant endometrium, however, of whatever origin, helps us but little in understanding the condition from the pathological point of view.

The transplantation of apparently normal tissue with its growth in the new situation requires some further explanation of its occurrence. Because the endometrium has special physiological reactions it has been argued that its pathology will be peculiar to itself. The corollary of this view is that pathological principles will be legion — there will be special processes for every organ in the body. This leads us into an untenable position.

On the other hand, in the hypothesis submitted in this paper, we are dealing with known, and for the most part, definite physiological phenomena and with fundamental and well known pathological principles.

The changes in the graafian follicle are well recognised, though some of the more abstruse manifestations still require elucidation. The process of atresia has been shown to be due to hormone activity, in some cases arising from a corpus luteum, but also in others from other parts of the body — particularly the anterior pituitary gland. It has also been shown by several experimental physiologists that the haemorrhage which occurs into the body of the ovary is the result of several factors, one of which is a hormone of the anterior pituitary gland.

The particular factors which are active in the human being are still undetermined, but, in the lower animals, I have ascertained experimentally that the anterior pituitary hormone may produce atresia and haemorrhage in the follicles and that, in many cases, the presence of the blood is associated with the development of an epithelial lining. This is to be discussed in another paper.

From this it would appear that the development of the epithelium is the result rather than the cause of the bleeding into the cysts.

give an appearance of glands. These are to be found particularly towards the end of longitudinally collapsed cysts. Another method of formation is a definite outgrowth of the epithelium into the surrounding tissue. That this is an active process seems certain from the observation that often the glands extend beyond the confines of the cyst wall, *i. e.* beyond the boundaries of the hyaline tissue. They may be traced for considerable distances and at times a gland space near the surface of the ovary may be shown, by serial section, to be actually continuous with a comparatively deep cyst.

Complications Due to Proximity to the Surface: It has been tacitly assumed by Sampson and other protagonists of the implantation hypothesis that the presence of glands near the surface of the ovary indicates an implantation of material on the surface of the organ. Actually the observations are to be explained as a rupture of primary ovarian cysts. The occurrence of glands derived from the immature follicle near the surface of the organ has been mentioned. In a similar manner glands arising from atretic follicles of greater development, in which epithelial activity is marked, occur near the surface and alterations of intracystic tension, such as rupture, affect the development of the lining (Figs. 12 and 13). Such rupture may be followed by a projection outwards of part of the inner wall (Fig. 14), and this gives a clue to the origin of the gland and stroma débris found on the surface of the organ. Portions of the original cyst wall can frequently be seen (Fig. 16). These features may be absent from one section, but serial sections should be made in such cases before conclusions are drawn.

COMMENT

These observations are only a few of those to be encountered in the examination of ovaries containing blood cysts. No doubt the extraordinary variations found in the structure of the walls of the cysts has been responsible for the opinion that the epithelium, which is such a constant feature, is the most important. That extreme variation of structure of the bodies of the ovary derived from the graafian follicle occurs normally may be determined by a study of a number of organs. These bodies have been insufficiently examined and their relationship to the blood cysts has not been appraised, and thus the significance of the epithelium has not been appreciated.

In the majority of current descriptions emphasis is laid on the

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DESCRIPTION OF PLATES

PLATE 74

FIG. 1. A typical graafian follicle showing an antrum folliculi, a cumulus containing the ovum and a well developed theca interna. The theca externa is comprised by those stroma cells which are arranged concentrically around the theca interna. S. = stratum granulosum. T. I. = theca interna. E. = theca externa. $\times 40$.

FIG. 2. A retrogressing follicle showing a well preserved theca interna (T. I.). The stratum granulosum (S.) is only one cell thick. $\times 35$.

FIG. 3. A small cystic atretic follicle which contained blood. The stratum granulosum (S.) is commencing to adopt an "epithelial" character. The follicular nature of the cyst is shown by the presence of the theca interna (T. I.). $\times 45$.

FIG. 4. A higher power view of a small atretic follicle containing blood. The stratum granulosum is represented by a single layer of flattened and cuboidal cells. The theca interna (T. I.) can still be recognised. $\times 65$.

FIG. 5. A collapsed atretic follicle showing a retrogressing stratum granulosum (S.) and a well marked theca interna (T. I.). There is a large extrafollicular haemorrhage (H.) in the left lower quadrant. Compare with Fig. 12. $\times 40$.

In a case recently observed (to be described elsewhere) typical cyclical menstrual bleeding occurred in ovarian cysts of proved ovarian origin. From this point of view it might be noted that the haemorrhage, often cyclical, which occurs in the non-epithelialised follicles and luteal bodies of the ovary has been disregarded by the authorities who uphold the "endometrial" hypothesis of the nature of the epithelium-lined blood cysts.

CONCLUSIONS

1. In the course of its development, the graafian follicle gives rise to a very varied series of structures, the advanced stages of which bear little or no morphological resemblance to the parent structure. A study of the intermediate stages is necessary for the demonstration of the relationship of the atypical forms to the original follicle.

2. Haemorrhage occurs so frequently into these structures as to be almost a normal phenomenon.

3. A heterotopic epithelial lining develops in the older cysts which contain blood. Since these older cysts, as stated, bear but little resemblance to the original follicles, the relationship has been overlooked.

4. These cysts are identical with those which, on account of the nature of the epithelium, have been described as "endometrial."

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PLATE 75

FIG. 6. Portion of the wall of a follicle containing blood. The remnant of the stratum granulosum is adopting an epithelial character. $\times 200$.

FIG. 7. Portion of the wall of a cyst of follicular origin showing the development of "glands" which were shown, by serial section, to be continuous with the cyst. $\times 50$.

FIG. 8. Portion of the wall of a luteal cyst occurring, amongst many others, in association with hydatidiform mole. The epithelial lining is composed of cuboidal cells. $\times 90$.

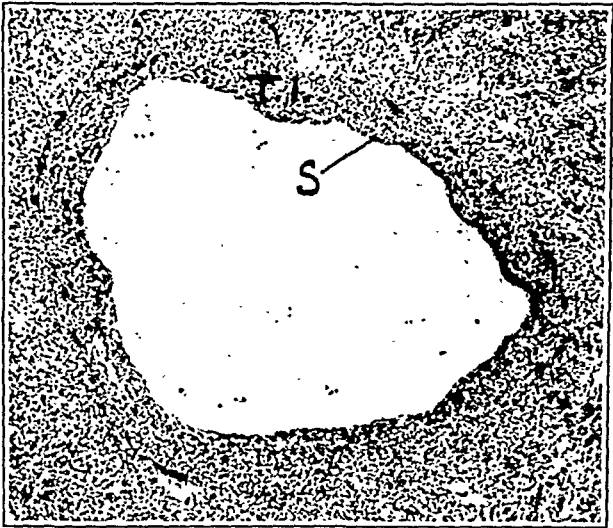
FIG. 9. A well developed epithelium-lined cavity which has arisen in an atypical atretic body, the greater part of which is composed of phagocytic cells (P.). $\times 25$.

FIG. 10. Portion of the wall of a cyst of luteal origin. There are numerous phagocytic cells which are in close association with the epithelium. $\times 100$.

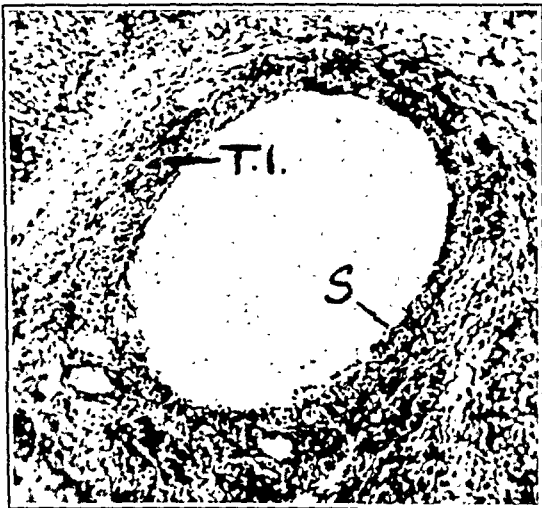
FIG. 11. Portion of the wall of a cyst of luteal origin showing the development of glands by crypt formation. $\times 50$.



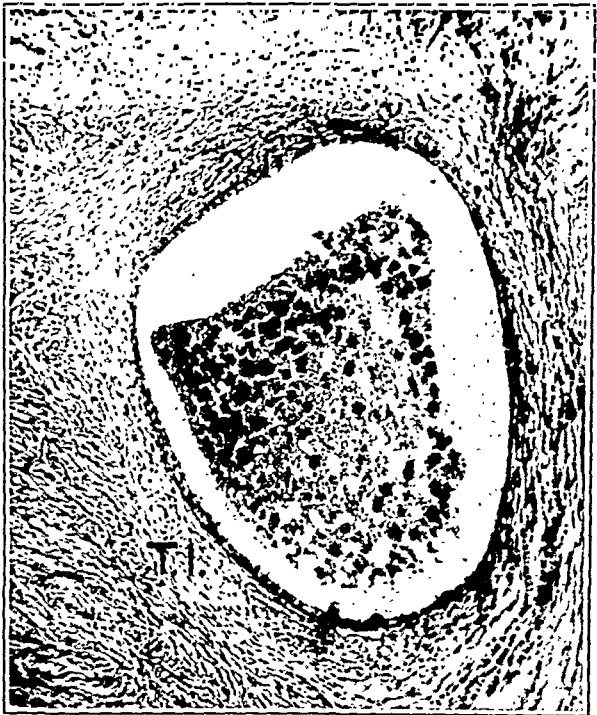
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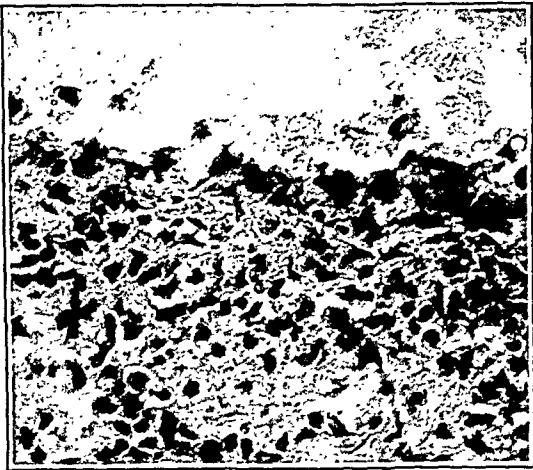
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PLATE 76

- FIG. 12. A cyst of follicular origin which has just ruptured onto the surface of the ovary. Note the projection of the epithelium on the deeper side of the wall and the swelling and haemorrhage into the subepithelial tissue. $\times 25$.
- FIG. 13. A small cyst near the surface of the ovary, of follicular origin (serial sections showed that other portions of the wall possessed characteristic stratum granulosum and theca interna). Haemorrhage has occurred into the subepithelial tissues. Compare with Fig. 5. $\times 40$.
- FIG. 14. A ruptured cyst in which the epithelium and subepithelial tissue of the deeper wall have formed a marked projection outwards. The glands present are due to an exaggeration of the process observed commencing in Fig. 13. $\times 15$.
- FIG. 15. An appearance regarded as being evidence for the implantation hypothesis. This tissue arises by disintegration of the projection shown in Fig. 14. In this case portion of the original cyst wall is to be seen at the left-hand edge (W.). $\times 12$.



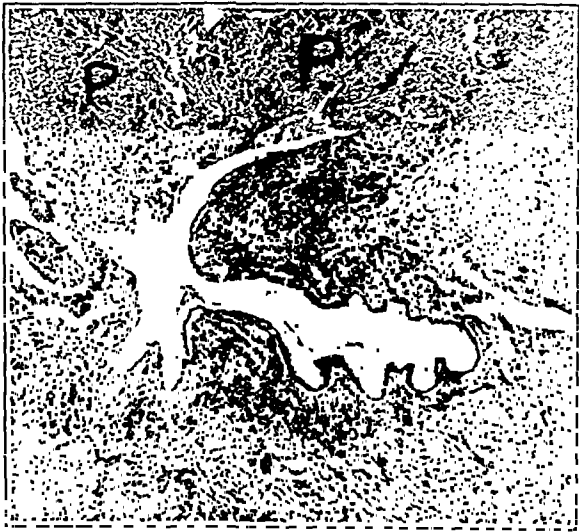
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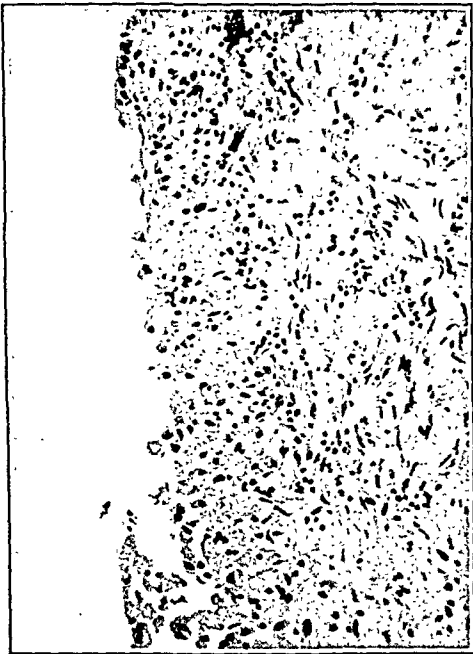
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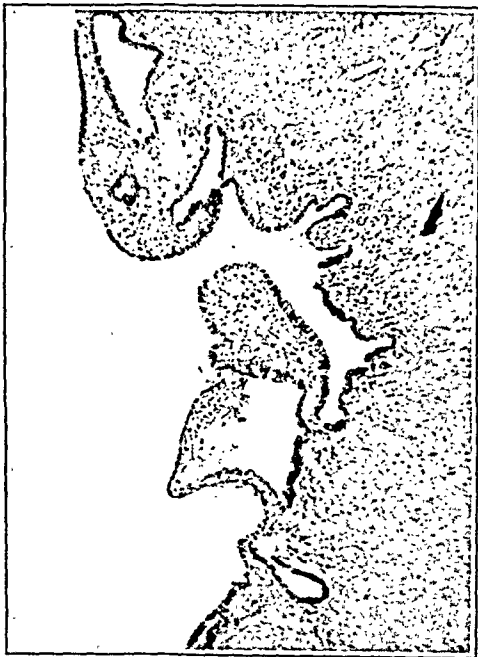
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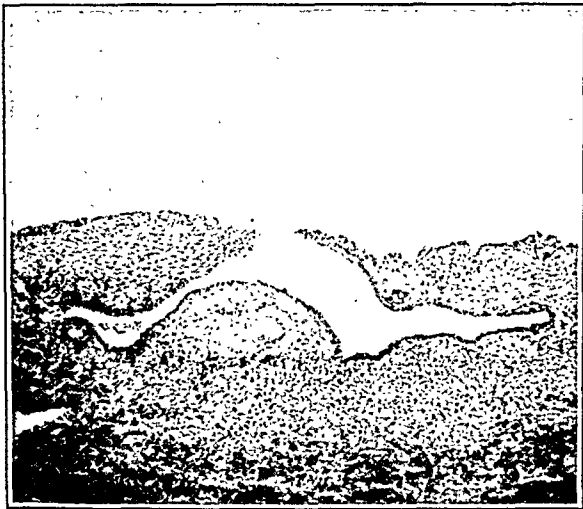
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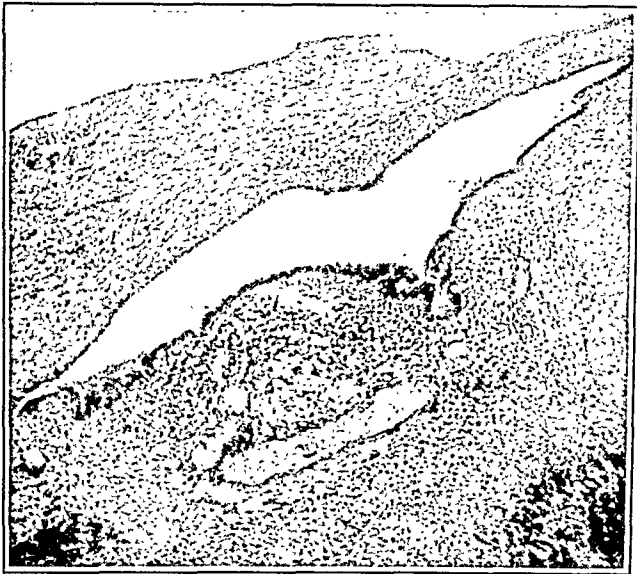
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King

Origin of Epithelium-Lined Blood Cysts



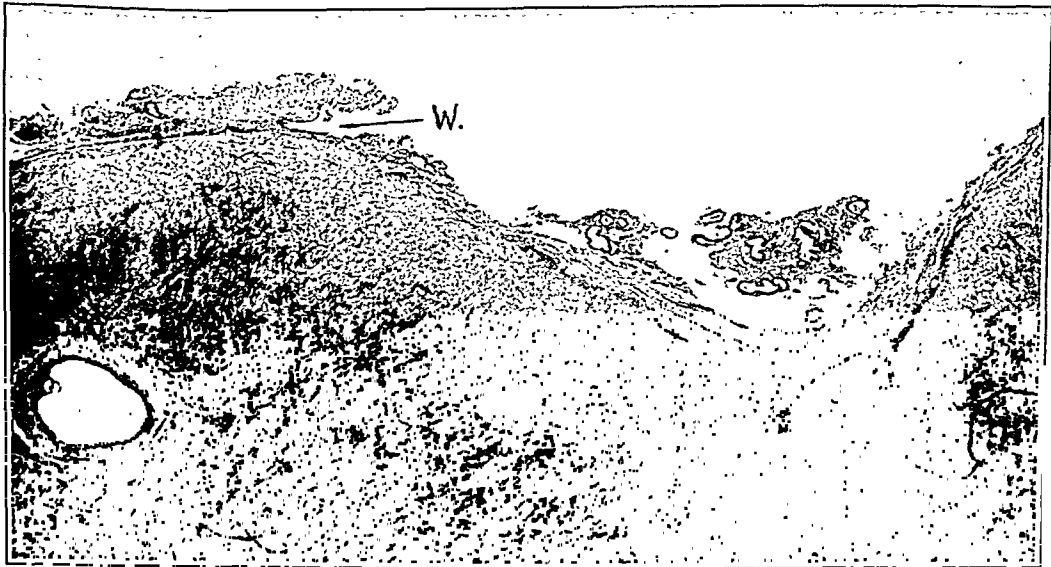
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tensis organism. The diagnosis in each case was dependent on clinical observation and a positive agglutination test. No organism was isolated.

After Evans,⁸ in 1918, emphasized the close laboratory similarity between the causative organism of Malta fever and the organism responsible for contagious abortion, the possibility of human infection with the latter organism was promptly raised by Evans, Huddleson and other investigators. Soon authentic reports of human cases of infection with the *Brucella melitensis* var. *abortus* began to make their appearance. The differentiation of the *Brucella* group of organisms by the bacteriostatic dye method of Huddleson^{9, 10} has clearly shown that *Brucella melitensis*, both porcine and bovine varieties, are pathogenic for man. The literature on human infection with these organisms has become more voluminous than that in which the original caprine variety of organism was implicated. The course of the disease, as well as the complications and sequelae, is similar even to the infrequency of meningitis. A review of the literature from 1918, the time the similarity was noted, to the present time has revealed only a single case due to the porcine organism, the pathology of which we propose to make the basis of this contribution. The clinical aspects of the case have been reported, in part, by Sanders.¹¹ No other instance of meningitis due to the bovine variety of organism has been found in the literature.

REPORT OF CASE

Clinical History: D. Y. (F-8521), a white, male, printer, 24 years old, entered the University Hospital on Oct. 10, 1931, and died on Nov. 3, 1931. He had been ill since Dec. 24, 1930. At that time, his right foot began to tingle and became numb. These sensations extended throughout the half of the body, even to the point of numbness of half of the tongue. He also developed aphasia. This attack lasted about thirty minutes. Following the first fifteen minutes of the numbness, a severe headache began in the temple and suboccipital regions, which lasted several hours. A number of similar attacks occurred later, except that they were initiated in an extremity other than the right leg. According to the mother, there had been four attacks of delirium. The patient was unable to recall anything about any of them. The first occurred in March, 1931, and lasted two hours. Lumbar puncture quite readily relieved his difficulties. He was admitted to Johns Hopkins Hospital the latter part of March as a brain tumor suspect, but no tumor could be located. However, evidence of intracranial irritation was present, and he was discharged. His illness was diagnosed encephalitis, or a low-grade meningitis. Two weeks after discharge, an organism of the *Brucella* group was isolated from the spinal fluid which had been obtained while the patient was in the hospital. Evidence of intracranial irritation had been

MELITENSIS MENINGO-ENCEPHALITIS *

MYCOTIC ANEURYSM DUE TO BRUCELLA MELITENSIS VAR. PORCINE

G. H. HANSMANN, M.D., AND J. R. SCHENKEN, M.D.

(From the Department of Pathology and Bacteriology, College of Medicine,
State University of Iowa, Iowa City, Ia.)

Contributions to our knowledge of Malta fever have been numerous since the discovery of the etiological organism by Bruce ¹ in 1887. A search of the voluminous literature on this subject reveals quite frequently most of the complications and sequelae of a long-continued intravascular infection. Meningeal infection with *Brucella* organisms is an exceptional observation in the history of the disease. The localization of *Brucella melitensis* var. *caprine*† in the meninges, as far as we are aware, has been conclusively proved only on three occasions. Hughes,² in his thorough study and review of the subject, mentions a patient who, after forty-five days of fever, had been afebrile for fifty-seven days, when symptoms of cerebral irritation appeared. *Brucella melitensis* var. *caprine* was isolated from the meninges postmortem, fifteen days after the onset of the cerebral symptoms. Lemaire ³ made a complete study of a non-fatal case in which he pointed out the similarity of melitensis meningitis and tuberculous meningitis. He either failed to locate the report by Hughes, or he did not accept it as a case of melitensis meningitis, because he believed that his was the first case of meningitis reported in which *Brucella melitensis* var. *caprine* was the causative organism. Desage and coworkers,⁴ having seen Lemaire's report, were in a position to make a diagnosis of melitensis meningitis on a patient who, after a number of consultations, had been given up to die, supposedly of tuberculous meningitis. Magnani ⁵ reported a series of observations on a possible case of melitensis meningitis. Roger ^{6,7} contributed nine clinical cases, six of his own and three from a text by Cantaloube, of probable infection of the meninges with the meli-

* Received for publication February 20, 1932.

† Because of geographic distribution of the cases, it is assumed that the organisms were from caprine sources.

December 15, and their sera agglutinated the *Brucella* organism in 1:1280 and 1:5120 dilutions, respectively. It was known before inoculation that their sera contained no agglutinins for the *Brucella* group.

Blood: The white blood cells varied from 9,200 to 15,900. There were 87 polymorphonuclear leucocytes, 10 lymphoid cells, and 3 large mononuclear cells.

Urine: There was nothing abnormal about the urine.

Spinal Fluid: The spinal fluid pressure was 52. On March 20, 1931, the fluid contained 300 cells, 36 per cent of which were lymphocytes. On May 7, 1931, there were 271 cells, only 12 per cent of which were polymorphonuclear leucocytes. Fluid obtained between Oct. 7, 1931, and time of the patient's death was markedly bloody in each instance. This bloody spinal fluid contained no reducing substance.

POSTMORTEM EXAMINATION

The spleen weighed 175 gm. With the exception of three small hemorrhages in the visceral pericardium, and obvious emaciation, the gross findings were confined to the central nervous system.

Liquid and clotted blood filled the subarachnoidal space at the base of the brain from the optic chiasma to the brain stem, inclusive. Hemorrhage completely surrounded the brain stem and was continuous with the blood clot which filled the cisterna magna, fourth ventricle, aqueduct of Sylvius, and third ventricle. Except for accumulations of blood covering both gyri and sulci over the occipital lobes, left Sylvian fissure and the tip of the left temporal lobe, the hemorrhage in the subarachnoidal space of the cerebral hemispheres was in the sulci. The leptomeninges of the anterior and central portions of both superior surfaces of the cerebral hemispheres contained a number of grayish white "tubercles." Most of these were found definitely clustered along vessels. Blood, which filled the entire spinal subarachnoidal space, obscured all evidences of a meningitis.

After the brain was fixed in 10 per cent formalin, the cerebral and cerebellar peduncles were severed so that the pons, medulla and fourth ventricle were obtained in one piece. This was sectioned coronally without disturbing the subarachnoid hemorrhage. An aneurysm which measured 9 by 6 by 6 mm. arose from the dorsal surface of the proximal portion of the basilar artery. The major portion of the sac lay superior to its point of attachment to the basilar artery. A short pedicle, containing a channel 1 mm. in diameter, connected the lumina of the sac and artery. The aneurysm, the basilar artery and the vertebral arteries, all lay embedded in blood. Examination of the cerebral hemisphere revealed somewhat dilated lateral ventricles and a granular ependyma. A small amount of

demonstrated by Doctor Sanders.¹¹ He found what he believed were meningo-cocci, and the patient received antimeningococcic therapy. After the patient's return home, he was treated with vaccine prepared from an autogenous and stock strain of the *Brucella* organism, with what appeared to be quite a satisfactory result. Trouble with his eyes (diplopia) when looking to either side, ears (some impairment of hearing and throbbing in his ears), and headaches had, however, not entirely disappeared and lumbar puncture was at times necessary for relief. On Sept. 30, 1931, he became nauseated, vomited, and for two days had a temperature of 105° F. A low-grade fever had persisted since that time. Physical examination on admission to the University Hospital Oct. 7, 1931, disclosed nothing abnormal, except for prominence of the eyes, a 20 pound loss of weight, and a somewhat unsteady gait.

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Laboratory Data:

Bacteriology: On March 20, 1931, Gram-negative organisms were found in the spinal fluid, which were interpreted as meningococci. A few days later a culture of the fluid examined at Johns Hopkins Hospital yielded a melitensis organism. On May 7, 1931, a *Brucella melitensis* var. *porcine* was isolated from the spinal fluid. On Oct. 26, 1931, five drops of uncentrifugalized spinal fluid on culture yielded no growth, while five drops of centrifugalized sediment yielded only seventeen colonies, which, when typed by Huddleson's bacteriostatic dye method, were proved to be *Brucella melitensis* var. *porcine*. On November 2, a similar organism was isolated from the spinal fluid. The fluid from the puncture on Oct. 26, 1931, was inoculated into two guinea pigs ("A" and "B"). The "B" pig was killed and autopsied after six weeks. Enlarged suppurating glands were found in the groin, the spleen was twice the normal size, and areas of necrosis were found in the liver and spleen. Organisms isolated from the lesions of this guinea pig proved to be *Brucella melitensis* var. *porcine*. The other pig, "A," was permitted to live and remain under observation.

Serology: On July 7, 1931, the patient's serum agglutinated the organism isolated from the spinal fluid, as well as a stock strain of *Brucella* in 1:160 dilution. On Oct. 22, 1931, the patient's serum agglutinated the stock strain of *Brucella* in 1:20 dilution, and the autogenous strain in 1:40 dilution. The spinal fluid agglutinated the stock strain in 1:5 dilution, and the autogenous strain in 1:10 dilution. The organism in the spinal fluid was agglutinated by known *Brucella* serum in a dilution of 1:1280. Guinea pigs "A" and "B" were bled on

tensis organism. The diagnosis in each case was dependent on clinical observation and a positive agglutination test. No organism was isolated.

After Evans,⁸ in 1918, emphasized the close laboratory similarity between the causative organism of Malta fever and the organism responsible for contagious abortion, the possibility of human infection with the latter organism was promptly raised by Evans, Huddleson and other investigators. Soon authentic reports of human cases of infection with the *Brucella melitensis* var. *abortus* began to make their appearance. The differentiation of the *Brucella* group of organisms by the bacteriostatic dye method of Huddleson^{9, 10} has clearly shown that *Brucella melitensis*, both porcine and bovine varieties, are pathogenic for man. The literature on human infection with these organisms has become more voluminous than that in which the original caprine variety of organism was implicated. The course of the disease, as well as the complications and sequelae, is similar even to the infrequency of meningitis. A review of the literature from 1918, the time the similarity was noted, to the present time has revealed only a single case due to the porcine organism, the pathology of which we propose to make the basis of this contribution. The clinical aspects of the case have been reported, in part, by Sanders.¹¹ No other instance of meningitis due to the bovine variety of organism has been found in the literature.

REPORT OF CASE

Clinical History: D. Y. (F-8521), a white, male, printer, 24 years old, entered the University Hospital on Oct. 10, 1931, and died on Nov. 3, 1931. He had been ill since Dec. 24, 1930. At that time, his right foot began to tingle and became numb. These sensations extended throughout the half of the body, even to the point of numbness of half of the tongue. He also developed aphasia. This attack lasted about thirty minutes. Following the first fifteen minutes of the numbness, a severe headache began in the temple and suboccipital regions, which lasted several hours. A number of similar attacks occurred later, except that they were initiated in an extremity other than the right leg. According to the mother, there had been four attacks of delirium. The patient was unable to recall anything about any of them. The first occurred in March, 1931, and lasted two hours. Lumbar puncture quite readily relieved his difficulties. He was admitted to Johns Hopkins Hospital the latter part of March as a brain tumor suspect, but no tumor could be located. However, evidence of intracranial irritation was present, and he was discharged. His illness was diagnosed encephalitis, or a low-grade meningitis. Two weeks after discharge, an organism of the *Brucella* group was isolated from the spinal fluid which had been obtained while the patient was in the hospital. Evidence of intracranial irritation had been

blood was present in the tips of the ventricular horns. A firm, laminated blood clot completely filled the third ventricle.

HISTOLOGICAL EXAMINATION

Histological examination of the viscera reveals considerable amounts of phagocytosed hemosiderin in the lungs and a few small focal areas of necrosis in the liver.

The nervous system presents changes due to a mild injurious agent. Both the pia and the arachnoid show various degrees of thickening, due largely to an inflammatory cell infiltration and a connective tissue proliferation. The inflammatory cells are largely lymphocytes and plasma cells, with a moderate number of large mononuclear cells. A few polymorphonuclear leucocytes are noted. Serial sections of several of the meningeal "tubercles" reveal that they are composed of irregular masses of hyalinized connective tissue, moderately infiltrated with chronic inflammatory cells. In one area, where the inflammatory cell infiltration is especially marked, necrotic tissue is present in which polymorphonuclear leucocytes are noted. In another similar area, the central portion is composed of large mononuclear cells surrounded by a dense collar of lymphocytes. Newly formed blood vessels are present in many of these inflammatory cell collections. It appears that these areas represent various stages in the formation of a "tubercle," from necrosis to connective tissue hyalinization.

Most of the meningeal vessels show a thickened adventitia, heavily infiltrated with inflammatory cells. There are all gradations between a thick collar of lymphocytes, in which very little adventitia is recognizable, to a thick collar of adventitial connective tissue heavily infiltrated with inflammatory cells. Several of the brain stem vessels show a partial replacement of the media by connective tissue with marked subintimal connective tissue proliferation. Inflammatory cells are present throughout the entire thickness of the wall in these instances. Where branches of the meningeal vessels pierce the cortex, a perivascular collar of inflammatory cells accompanies the vessels. Many of the cortical and subependymal vessels show perivascular collections of lymphocytes. This phenomenon is virtually confined to these two areas of brain substance. There are two small areas of softening in the brain stem. One submeningeal area shows vacuolization of cells, edema,

December 15, and their sera agglutinated the *Brucella* organism in 1:1280 and 1:5120 dilutions, respectively. It was known before inoculation that their sera contained no agglutinins for the *Brucella* group.

Blood: The white blood cells varied from 9,200 to 15,900. There were 87 polymorphonuclear leucocytes, 10 lymphoid cells, and 3 large mononuclear cells.

Urine: There was nothing abnormal about the urine.

Spinal Fluid: The spinal fluid pressure was 52. On March 20, 1931, the fluid contained 300 cells, 36 per cent of which were lymphocytes. On May 7, 1931, there were 271 cells, only 12 per cent of which were polymorphonuclear leucocytes. Fluid obtained between Oct. 7, 1931, and time of the patient's death was markedly bloody in each instance. This bloody spinal fluid contained no reducing substance.

POSTMORTEM EXAMINATION

The spleen weighed 175 gm. With the exception of three small hemorrhages in the visceral pericardium, and obvious emaciation, the gross findings were confined to the central nervous system.

Liquid and clotted blood filled the subarachnoidal space at the base of the brain from the optic chiasma to the brain stem, inclusive. Hemorrhage completely surrounded the brain stem and was continuous with the blood clot which filled the cisterna magna, fourth ventricle, aqueduct of Sylvius, and third ventricle. Except for accumulations of blood covering both gyri and sulci over the occipital lobes, left Sylvian fissure and the tip of the left temporal lobe, the hemorrhage in the subarachnoidal space of the cerebral hemispheres was in the sulci. The leptomeninges of the anterior and central portions of both superior surfaces of the cerebral hemispheres contained a number of grayish white "tubercles." Most of these were found definitely clustered along vessels. Blood, which filled the entire spinal subarachnoidal space, obscured all evidences of a meningitis.

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phagocytosed hemosiderin, inflammatory cell infiltration and snarled glia. The other, located 2 mm. from the meninges, is a more recent lesion composed mostly of large mononuclear cells, lymphocytes and cellular debris. A hyaline thrombus is noted in a small vessel.

The ependyma is irregular, due to rounded elevations and crypt-like depressions. Proliferation of ependymal cells, especially over the elevations, is noted. In some areas complete replacement of the ependyma by inflammatory cells and cellular debris is present. There is a small subependymal area of focal necrosis.

Inflammatory cells are present in the perineureii of the nerve roots. The central portion of one nerve funiculus is hydropic and the periphery shows condensation of nerve fibers, some of which are swollen.

DISCUSSION

The spinal fluid is the important consideration in the study of melitensis meningitis. Lemaire made much of the increase of sugar in the spinal fluid. Desage and coworkers found opposite results. All are agreed that a mononuclear pleocytosis of the spinal fluid, the number of cells being frequently below 100 per cmm., is a very important consideration in the study of the spinal fluid of these cases. This has held true in our case, with the exception of the possibility of a polymorphonuclear preponderance early in the disease. The information on the counts of proved cases is tabulated below.

Author	Cells per cc.	Polymorphonuclear leucocytes	Mononuclear cells	Large mononuclear leucocytes	Small mononuclear leucocytes	Lymphocytes
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
3	25	..	100	100
3	225	..	100	100
3	180	2	98	98
4	50	16	84	43	27	14
4	60	12	88	32	38	18
11	300	64	36	36
11	70
11	271	12	88

The central nervous system involvement may be the first and only clinical manifestation of the disease. Of the proved and probable cases, eleven were preceded by the septicemia symptoms characteristic of Malta fever; in three the disease was manifested only by a

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the feeding of a limited number of organisms, or by the diffusion of the bacterial poisons from the localized process into the spinal fluid, or by both mechanisms, a chronic meningitis may be produced. These are atypical cases, but must be kept in mind. Here we consider diffuse infection of the meninges by widespread distribution of the causative organism in the subarachnoidal space, which results in a chronic meningitis. The *Hemophilus influenza* group of organisms, the *Treponema pallidum*, the *Mycobacterium tuberculosis*, and the *Torula histolytica* are well known as causative agents in chronic meningitis. To this group we must now add meningitis due to the organism of the Brucella group. Involvement of the meninges by members of the *Hemophilus influenza* group is easily recognized. The symptoms are more acute than in any of the other four types mentioned. The inflammatory cell in the spinal fluid is the polymorphonuclear leucocyte. The course of the disease is usually that of a basilar meningitis of about a month's duration before resulting in death. The tuberculous meningitis is very similar, except that evidence of meningeal irritation is not so severe. Headache is sometimes a distressing symptom. The course of the disease averages about six weeks in duration, and usually terminates in death. Mononuclear cells usually predominate in the pleocytosis of the spinal fluid, but counts of 75 per cent of polymorphonuclear leucocytes may obtain in tuberculous meningitis. Torula infection is accompanied by severe headaches and at times marked evidence of intracranial pressure. So marked is the intracranial pressure that the slightly stiff neck and pleocytosis of the spinal fluid are disregarded, and brain tumor is erroneously diagnosed. The cells in the spinal fluid are chiefly mononuclears and the yeast-like organisms may be mistaken for cells in the fluid, unless one is on guard. Torula meningitis proves fatal in about six months. The symptoms of syphilitic meningitis and melitensis meningitis are frequently nothing more than a severe headache. Both diseases tend to recover. The melitensis meningitis is associated with startling, but evanescent, central nervous system symptoms such as hemiplegia, paraplegia, aphasia, diplopia and so on. There is a mononuclear increase in the spinal fluid in both instances, which may rise to several hundred cells. It is hardly worth while to go more extensively into the clinical differentiation of these conditions. Enough has been said for an intelligent approach to a definitive diagnosis through an accurate study of

meningeal irritation. The central nervous system involvement as the initial phenomenon of the disease renders the spinal fluid study extremely important. The blood may contain no agglutinins and the association of a meningitis with Malta fever may be overlooked. But the finding of the organism in the spinal fluid, the testing of its pathogenicity on guinea pigs, and the differentiation of the organism according to the bacteriostatic dye method of Huddleson, which constitute the only way to make certain a diagnosis, may be regularly accomplished. In no individual case is it safe to assume that a patient is suffering from Malta fever or melitensis meningitis on the basis of an agglutination titer alone. Many people have serum agglutinins for the Brucella group, with no symptoms. Here, as in other conditions, the agglutination reaction may lead one to an incorrect diagnosis, with disastrous results. We are too prone these days to accept an agglutination result as the determining factor in the diagnosis of the current illness. No procedure is comparable in trustworthiness to the isolation and identification of the organism, when this is possible. The isolation of the organism is relatively simple in melitensis meningitis if we keep in mind the fact that only a few organisms are present in a cubic centimeter of spinal fluid. It is general experience that the use of 10 cc. of inoculum has regularly yielded positive results. Our only failure in obtaining the organism was accounted for by too meager an inoculation. Desage and co-workers, and Lemaire, strongly emphasize this point.

The headache, the diplopia, the transitory paralyses, and the throbbing in the ears are outstanding in the course of the disease. Convulsions may occur. Desage used X-ray therapy successfully to relieve a paraplegia which he believed was due to adhesions of the spinal cord. Recovery was rapid following this therapy. The case report by Hughes mentions congestion as the only morbid anatomical change in the central nervous system. Our case showed no pathology which would cause a paraplegia, as Desage assumed. Other untreated cases, as well as our own, recovered from these paralyses equally as satisfactorily as the case described by Desage. It is conceivable that the encephalitis, and particularly the edema of nerves with swelling of nerve fibers, as evidenced in our case, might lead to the evanescent symptoms observed in this disease.

Any organism may be localized in the pia-arachnoid or in the larger vessels at the base of the brain for a long period of time. By

DESCRIPTION OF PLATES

PLATE 77

- FIG. 1. Photograph of the ventral surface of the brain. Note the subarachnoid hemorrhage, especially over the base. The brain stem is completely encircled with blood. The basilar artery is not visible.
- FIG. 2. Drawing of the superior cerebral surfaces. Note the clusters of "tubercles" located largely along vessels in the sulci.
- FIG. 3. Photograph of a coronal section through the pons and fourth ventricle. Note the basilar artery and its aneurysmal sac embedded in blood clot. The fourth ventricle is filled with blood clot.
- FIG. 4. Same as Fig. 3, after removal of fourth ventricle blood clot. Note the blood-stained ependyma. (Arrow 1 indicates basilar artery; Arrow 2 indicates aneurysm.)
- FIG. 5. Photograph of coronal section through pons and fourth ventricle superior to Fig. 3. Note the superior extension (2) of the sac between the artery (1) and the pons.

the blood, and particularly of the spinal fluid, from an immunological and bacteriological standpoint.

SUMMARY AND CONCLUSIONS

1. A case of melitensis meningo-encephalitis is reported, with a review of the related literature.
2. A mycotic aneurysm due to *Brucella melitensis* var. *porcine* was the immediate cause of death.
3. According to the literature and in our own case as well, isolation of the organism from the spinal fluid is relatively simple, if approximately 10 cc. of inoculum is used.
4. Headache and evanescent paralyses are important central nervous system manifestations, and mononuclear pleocytosis is an outstanding feature of the spinal fluid.

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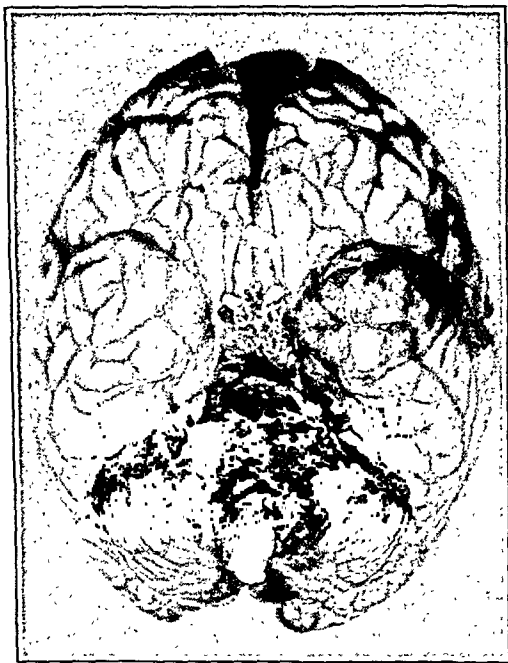
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PLATE 78

FIG. 6. Photomicrograph of cerebral cortex. Note the dense perivascular lymphocytic infiltration. $\times 200$.

FIG. 7. Photomicrograph of meninges and submeningeal area of necrosis of the brain stem. Note the vacuolization and glial replacement. Large "spider" cells are present. The pia is markedly thickened and heavily infiltrated with inflammatory cells. $\times 200$.

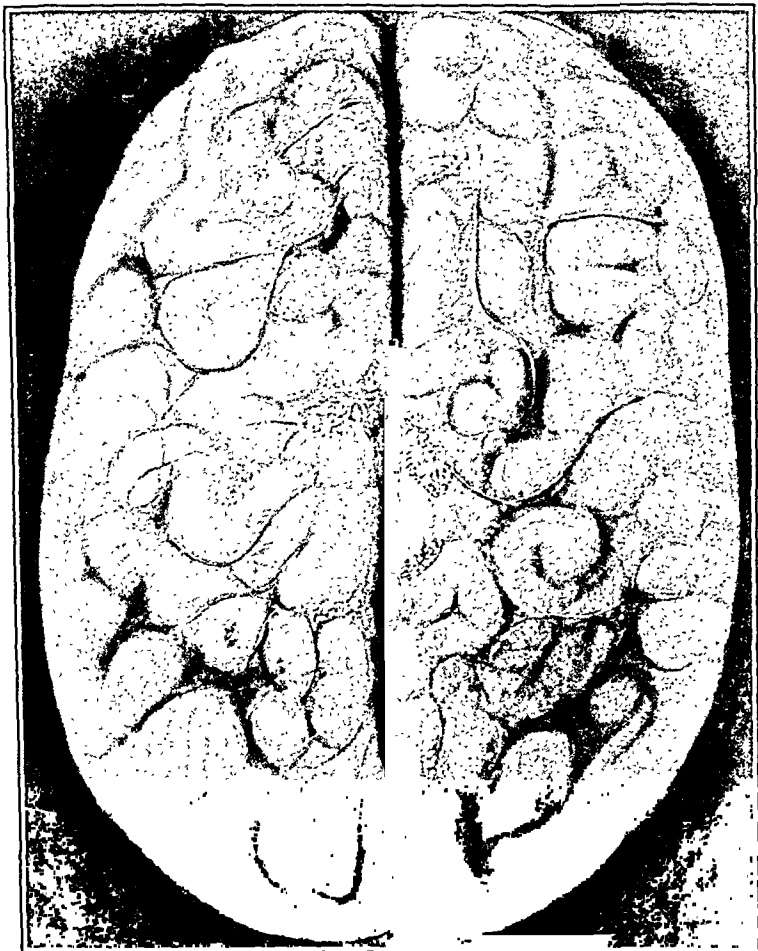
FIG. 8. Photomicrograph of area of necrosis in brain stem. Note the large numbers of macrophages in the area of tissue liquefaction. $\times 200$.



1



3



2



4

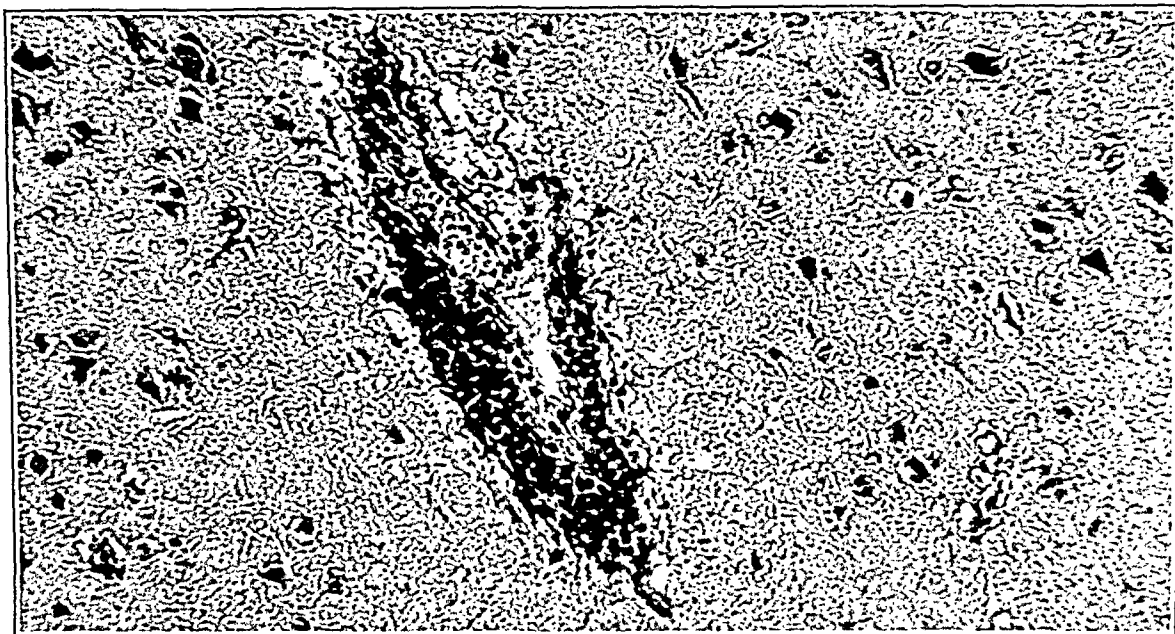


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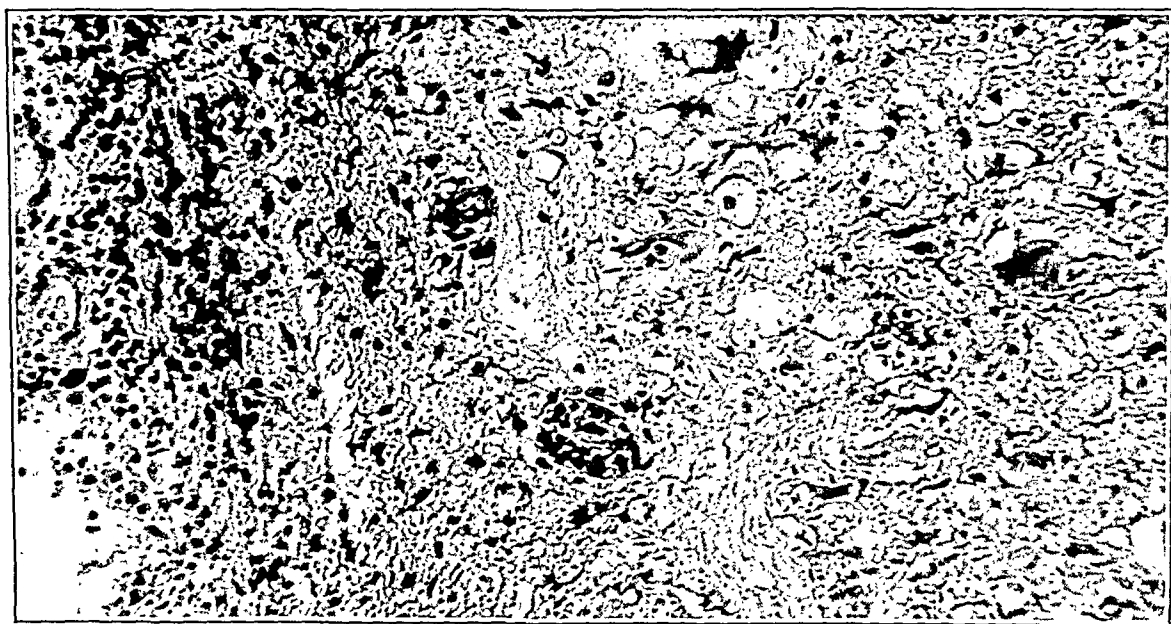
PLATE 79

FIG. 9. Photomicrograph of meningeal vessel showing a dense collar of lymphocytes. $\times 200$.

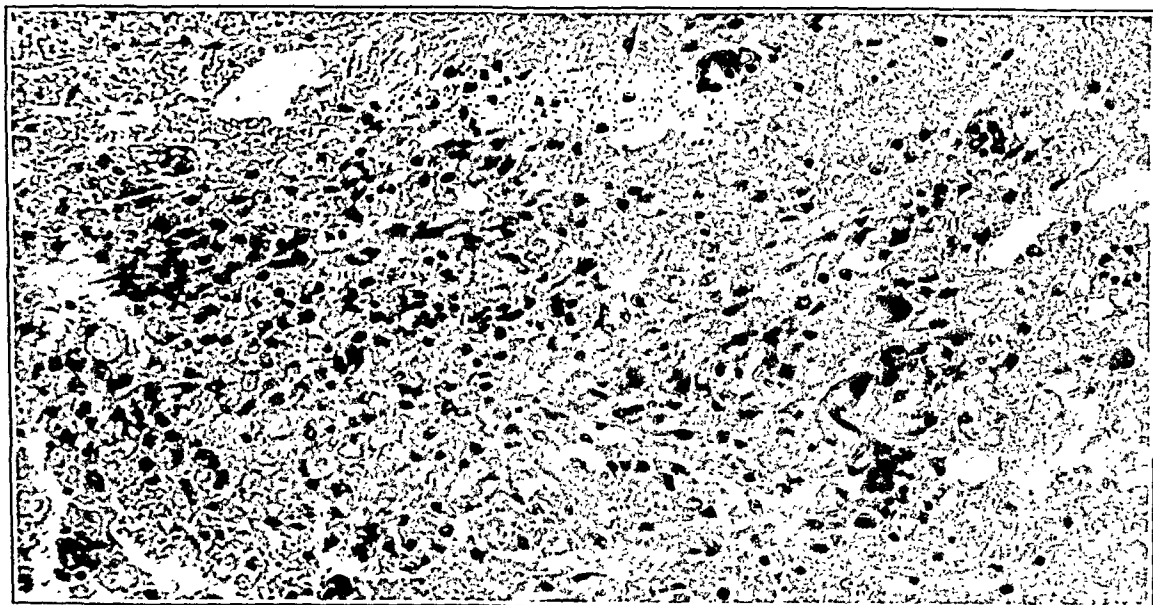
FIG. 10. Photomicrograph of a small brain stem artery. Note the increased adventitial connective tissue heavily infiltrated with inflammatory cells and the marked destruction of the media with connective tissue replacement. Definite endarteritis is present. $\times 200$.



6



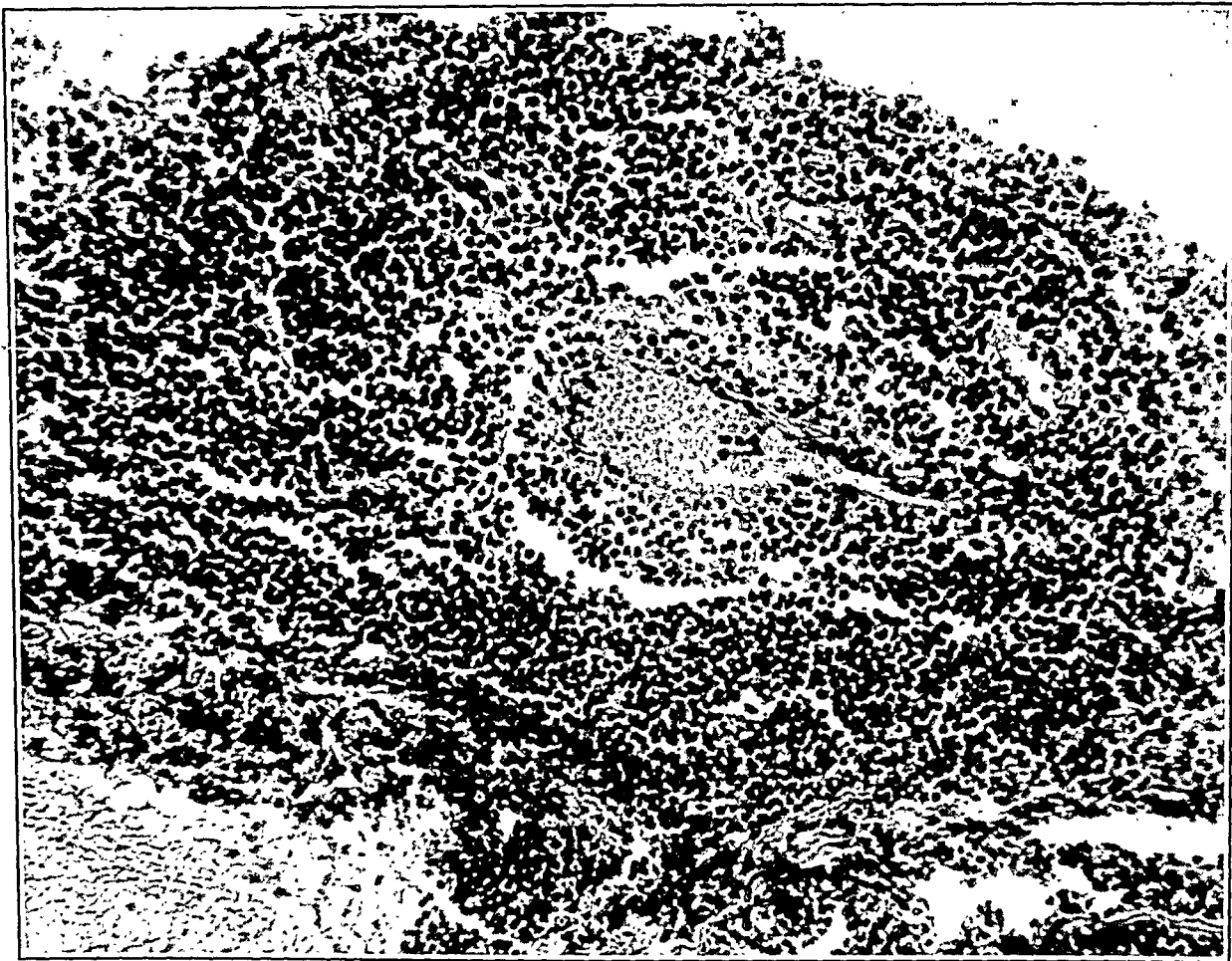
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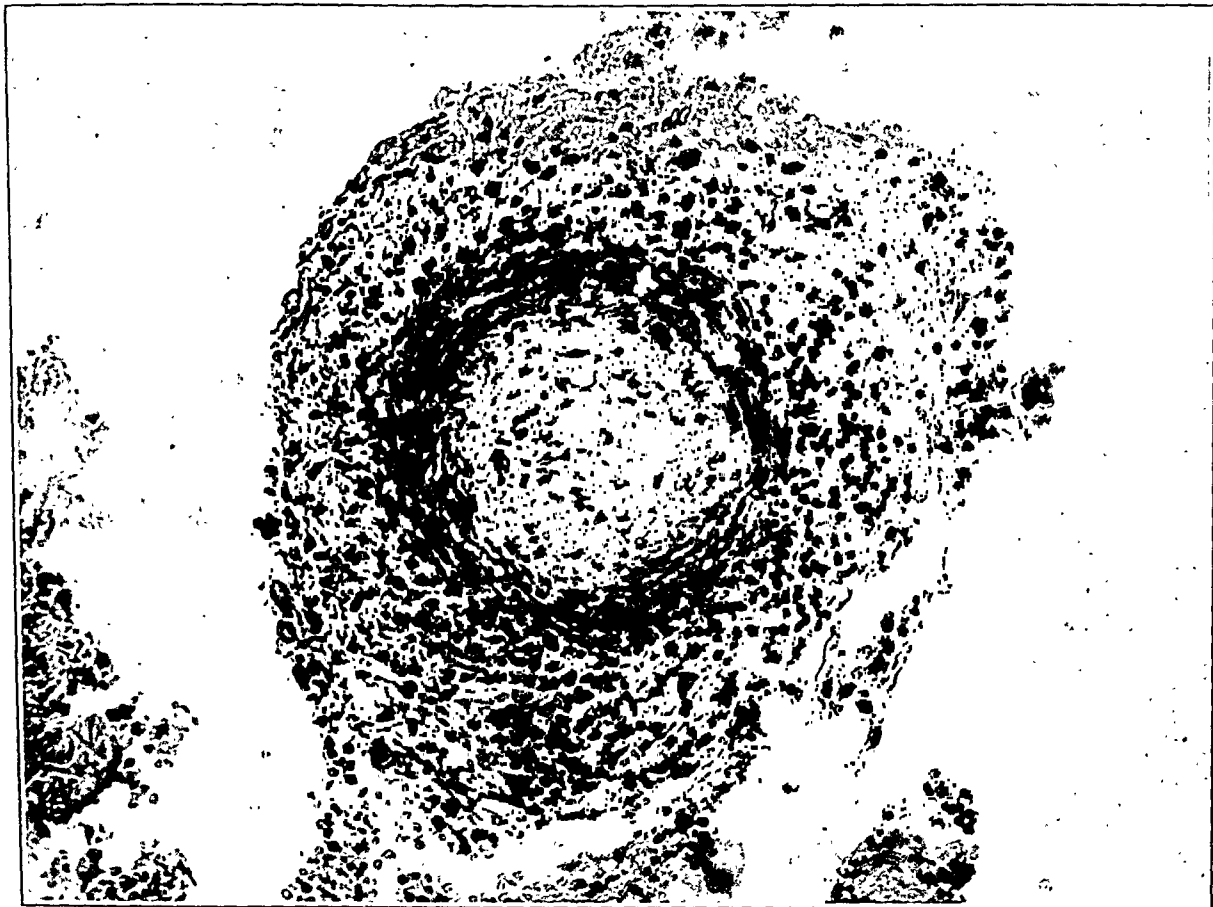
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PLATE 80

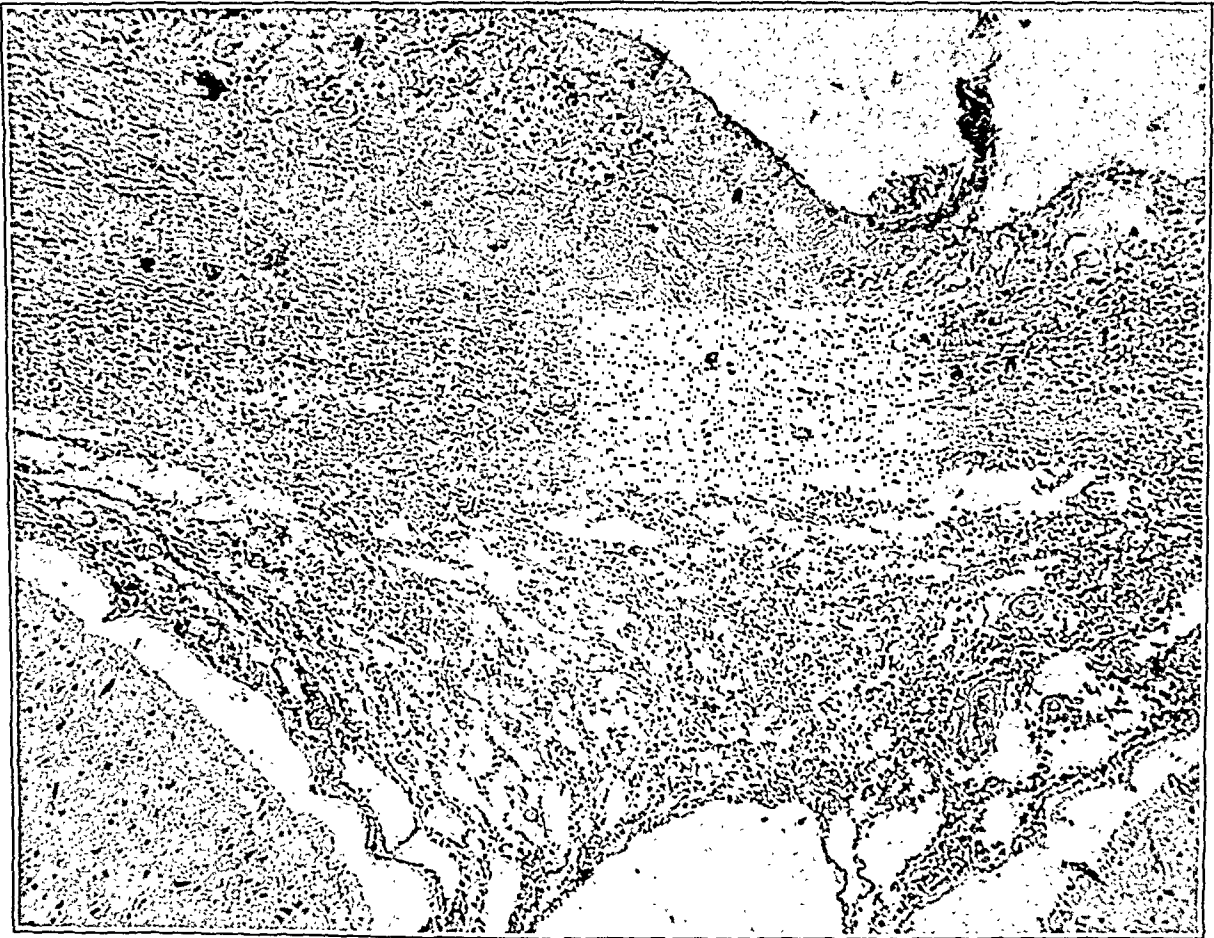
- FIG. 11. Photomicrograph of the leptomeninges through a "tubercle" area. Note the marked thickening, inflammatory cell infiltration, connective tissue proliferation, the somewhat acellular hyaline area representing the tubercle, and the pia-arachnoid fusion. $\times 55$.
- FIG. 12. Photomicrograph of a funiculus of a nerve root. Note the central hydropic area with a peripheral condensation of fibers. $\times 200$.



9



10



11



12

had undergone operations for resection of the primary tumor. Unfortunately Simmons does not describe the histological structure of the nodules in the lungs. In the instance reported by Ewing²⁴ of an adamantinoma with metastases to the lung, the histological structure of the metastatic deposits was that of a malignant growth.

The following instance of an adamantinoma is reported because of the unusual metastatic lesion in the lung.

CASE REPORT

An adult of 38 years was admitted to Montefiore Hospital on Sept. 3, 1930, with the chief complaint of a productive cough. Twenty-one years prior to the present admission, he first noted a lump in the right upper jaw. At that time excision of the tumor was attempted and five subsequent operations were performed, due to recurrence of the local lesion. In 1925 a small, soft, reddish tumor of the right upper jaw in the scar of a previous operation was removed at the Haggard Clinic in Nashville, Tenn. The area was cauterized and radium needles were implanted in the surrounding tissues. Pathological examination at that time showed the tumor to be an adamantinoma. A few months later a radical excision was performed. At the time of the operation the antrum was filled with a brain-like material which penetrated into the nasal cavity and involved the septum. Intensive radium treatment was given and the local condition healed. For a period of five years following this operation there was no evidence of recurrence locally. Two years ago, however, after an attack of "dry pleurisy," which confined the patient to bed for three weeks, he developed a productive cough with occasional blood-streaked sputum. About a year ago he complained of progressive weakness, anorexia, marked loss of weight and pain in the right hip. An X-ray of the chest taken at the Lebanon Hospital revealed a dense mass in the lower lobe of the right lung, extending from the sixth rib to the diaphragm and obscuring the right half of the latter. In the base of the left lung a circumscribed, moderately dense nodule the size of a large marble was also noted. Nothing abnormal was revealed in the X-rays of the right hip, pelvis and vertebral column. He was transferred to Bellevue Hospital where he received X-ray therapy for the lung condition.

At the time of admission to Montefiore Hospital, examination of the patient revealed a transverse and vertical scar on the right cheek. There was evidence of a partial resection of the hard palate and the right upper jaw, with a fairly large cavity in the roof of the mouth on the right side, measuring about 3 by 3 by 3 cm. opening into the nasopharynx. There was no evidence of recurrent growth. On examination of the chest there was a slight retraction of the fourth, fifth and sixth intercostal spaces over the right anterior wall. Expansion was slightly limited on the right side. Vocal fremitus was increased anteriorly from the third intercostal space downward and markedly diminished from the level of the eighth dorsal vertebra to the base. There was flatness posteriorly from the apex of the axilla to the seventh dorsal vertebra. Posteriorly the breath sounds were entirely absent below the seventh dorsal vertebra. Examination of the rest of the body revealed no abnormalities.

Nine months after admission the patient developed a septic temperature and signs of an empyema on the right side. A thoracotomy was performed and three

AN INSTANCE OF ADAMANTINOMA OF THE JAW WITH METASTASES TO THE RIGHT LUNG *

JEFFERSON VORZIMER, M.D., AND DAVID PERLA, M.D.

(From the Laboratory Division, Montefiore Hospital, New York, N. Y.)

Since Guzack ¹ in 1826 described the first case of cystic tumor of the lower jaw,† and Falkson ² in 1879 gave the first full account of this condition, at least 130 cases of adamantinoma have been recorded.^{3-20†} Adamantinomas are tumors probably arising from the so-called "paradental epithelial débris," first described by Malassez.²¹ The enamel organ develops by a downward growth of the gingival epithelium. All of the epithelium of the enamel organ, except its internal layer, undergoes atrophy and is absorbed. Occasionally these cells persist and it is believed that they are the histogenetic precursors of adamantinomas. The structure of the adamantinoma closely resembles the embryonal enamel organ.

Most investigators consider the adamantinoma a benign tumor.²² It may, however, undergo malignant transformation, and where this occurs metastases having the histological structure of the malignant growth have been reported (Aschoff,²³ Ewing,²⁴ Kaufmann,²⁵ Heath,²⁶ Krompecher,²⁷ and others). Such malignant transformation develops usually after several local recurrences, following repeated excisions. From an analysis of the literature it is apparent that metastases from an adamantinoma are extremely uncommon. Eve ²⁸ in 1883 described an unusual instance of a cystic tumor of the lower jaw of thirteen weeks' duration in a woman 60 years of age, who died of postoperative bronchopneumonia. Metastases to the lumbar lymph nodes were noted. Simmons ²⁹ in 1927 reported two cases of adamantinoma with metastases. In one instance the metastases were in the regional lymph nodes, appearing fourteen years after the onset of the disease. In the second instance metastases were present in the glands of the neck and in the lungs. In both instances the patients

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† Sculter, cited by Albarran, J., *Rev. de chir.*, 1888, 8, 429, is said to have described cysts of the jaw in 1654.

‡ Coryllos (ref. 3), gives a complete bibliography of the literature on this subject up to the year 1910.

cavity. The right lung was closely adherent over the lower lobe posteriorly, laterally, and at the base.

The heart was not enlarged, and weighed 260 gm. The parietal pericardium adjacent to the lower lobe of the right lung was closely adherent to it. There was a moderate amount of epicardial fat which was well defined from the musculature. The myocardium was reddish brown in color and firm in consistence. The right auricle and ventricle presented no abnormalities. An occasional small, yellowish patch was seen through the endocardium in the right ventricle. The endocardium of the left auricle was somewhat opaque and grayish. Attached to the auricular surface of the aortic cusp of the mitral valve, by a broad base which measured 1.5 cm. in diameter, was a lobulated mass about the size of an olive. It had a somewhat smooth, greenish gray surface to which was adherent some dark red, clotted blood. The mass was fairly firm, somewhat elastic and quite friable. Most of the chordae tendineae of the aortic leaflet were markedly thickened and on the aortic surface of the aortic leaflet directly opposite the polypoid mass there was a punched out ulceration about 1 cm. in diameter. Its edge was fairly smooth and was covered in places by a small amount of grayish, friable material. The base of this ulcer around the periphery could be probed for a distance of about 8 to 10 mm. into the polypoid mass. In the central portion at a depth of 2 to 3 mm. a greenish gray mass similar to that of the polyp was present. This material was adherent to the lower margin of the ulceration. Clotted blood was removed from the ulcerated region. The tips of the papillary muscles were firm, and grayish white in appearance. There were many grayish streaks seen in the endocardium of the left ventricle.

The upper and middle lobes of the right lung were crepitant throughout. The pleura was smooth and glistening and presented no abnormalities. On section, the surface was reddish gray, mottled with anthracotic pigment with an occasional darker red area of congestion. A large amount of well aerated serous fluid and blood exuded on pressure. Bronchi and pulmonary vessels of these lobes presented no abnormalities.

The entire posterior part of the lower lobe was torn on removal, with marked destruction of the parenchyma. There was a large area in the upper anterior portion which was firm and non-crepitant. On section, the lung pattern was completely distorted and the alveolar

subpleural abscess cavities were drained. Following this his temperature dropped to normal. On histological examination of the tissue removed at operation a diagnosis of metastatic adamantinoma was made. Three months later he had a hemoptysis of 5 oz. of bright blood and continued to have blood-streaked sputum. A bronchoscopic examination, which was performed at that time, revealed a growth blocking the bronchus leading to the right lower lobe, from which bright red blood was oozing. Section from the tumor revealed the structure of an adamantinoma. Two days later he developed chills and a septic temperature, and for the first time a systolic and diastolic murmur were heard over the apex of the heart. There was no evidence of cardiac enlargement. An acneiform eruption then appeared over the chest and back. A culture of *Streptococcus viridans* was isolated from the blood stream. The patient became irrational and died.

Laboratory data showed no important findings other than the positive blood culture. Roentgenological examination of the maxillae and mandibles revealed no gross pathological changes other than those caused by the operative excision of the right upper maxilla. The mass in the right lung decreased slightly in size following a course of radiotherapy but subsequently again increased in size. The small nodule in the left base, after slightly increasing in size, completely disappeared. Roentgenological examination of the skull and skeletal system revealed no abnormalities.

AUTOPSY REPORT

Anatomical Diagnoses: Adamantinoma of the right upper jaw (postoperative) with metastases to the lower lobe of the right lung; polypoid thrombus attached to the auricular surface of the mitral valve with perforation of the leaflet; *Streptococcus viridans* septicemia with localization of the streptococci on the thrombus; splenomegaly; chronic passive congestion of the viscera; bronchopneumonia of the right lower lobe.

The body was that of a well developed, well nourished, adult white male about 165 cm. in length. There were a few petechiae in the inferior conjunctival sac of the right eye. There was anemia of the conjunctivae and lips, and cyanosis of the nail beds. There were no palpable lymph nodes in the neck, axilla or groin. Extending backward from the right angle of the mouth for a distance of about 2 inches there was a healed scar. The right cheek sagged inward as most of the maxilla and antrum wall had been removed from the right lateral incisors backward. The mucous membrane of the right cheek presented a healed scarred appearance. There was no evidence of ulceration. On opening the chest and abdomen, the dome of the diaphragm reached the third intercostal space on the right side and the fourth rib on the left. There was no fluid or air in either pleural

leukocytes. Occasionally in some areas the polymorphonuclear leukocytic infiltration predominates.

The thrombus on the auricular surface of the aortic leaflet of the mitral valve shows tissue composed of necrotic material. Large masses of bacteria and fibrin are present and the base of the polypoid mass contains very dense cellular connective tissue which is partly hyalinized. The tissue is markedly vascularized with small capillaries which are congested. There are numerous round cells and polymorphonuclear leukocytes.

Sections through portions of the lung uninvolved with tumor show extensive edema and congestion of the vessels. In some areas alveoli are filled with large mononuclear cells which contain brown pigment. Occasionally these cells are fused to form giant cells with nuclei arranged eccentrically.

An area of consolidation of the upper portion of the right lower lobe shows extensive fibrosis, atelectasis and atypical epithelial proliferation in some of the infundibula, and alveoli in the fibrotic areas. The wall of a bronchus shows very marked dilatation of the lumen (bronchiectasis), striking inflammation of the wall with extensive infiltration of round cells, polymorphonuclear leukocytes and newly formed blood vessels. One of the large blood vessels shows some thickening of the wall with adherent thrombotic material in the lumen. Areas of bronchopneumonic exudate are present.

Section through tumor of lung shows large irregular islands of tumor cells separated by dense fibrous connective tissue. These tumor masses have an outer layer composed of elongated cylindrical epithelium arranged in a vertical orientation to the surface. Occasionally there are two layers of cells of this type. The rest of the tumor masses are composed of spindle-shaped and round cells in a very loose meshwork of fine fibrillar connective tissue. The cytoplasm of the cells within the core of the nodules is scanty. An occasional fine process can be seen definitely radiating outward, communicating with similar processes in other cells (star-shaped cells). In the center of some of the islands within the core of the tumor mass the cells are arranged in a whorl resembling the beginning of epithelial pearl formation. An occasional small, cyst-like space is present within the tumor masses. There is no evidence of a malignant transformation. There is a striking similarity between the histological structure of this tumor tissue and that of the enamel organ of a four month fetus.

portions were markedly diminished in amount and obscured. The bronchi were dilated and very prominent. In the lower portion of the lobe they formed large bronchiectatic cavities with smooth-ribbed walls and with bands running through them. The whole bronchial tree, wherever it was intact, was plugged with a cast composed of fairly firm, somewhat friable, grayish white tumor tissue. In some areas this plug measured 2 to 2.5 cm. in diameter. Although for the most part this cast lay free in the bronchi, in some areas the walls of the bronchi seemed to be invaded. The cartilages of the larger bronchi were somewhat thickened in some areas and in others thinned out. There was an occasional large patch of the remaining alveolar portion which was completely replaced by tissue similar to that found in the bronchi. This tissue seemed to invade the bronchial wall and in places the mucosal surface was raised by irregular, grayish white patches. The upper anterior portion of this lobe was completely consolidated. On section the surface was yellowish gray and translucent, with areas of necrosis and many small cavities surrounded by reddish zones.

The left lung was similar to the upper and middle lobes of the right lung, with the exception of a small nodule found in the middle of the lower lobe. This nodule was about 1.5 cm. in size, well defined from the rest of the parenchyma, and seemed to be composed of firm yellowish material. There was a small amount of fibrosis around it.

The spleen weighed 220 gm. and measured 14.5 by 9 by 3.5 cm. The organ was enlarged, soft and somewhat flabby in consistence. The capsule was bluish gray in color and presented no abnormalities. On section the surface was purplish red in color with many irregular areas of hemorrhage and congestion. The corpuscles were indistinct but the trabeculae were quite prominent. The pulp scraped with ease on the edge of the knife.

There were no other abnormalities found in the rest of the viscera, other than chronic passive congestion.

HISTOLOGICAL EXAMINATION

On microscopic examination the heart shows fragmentation of muscle fibers, cloudy swelling, small areas of perivascular edema and round cell infiltration, foci of cellular infiltration between muscle fibers composed of round cells and occasional polymorphonuclear

3. The growth of the tumor in the bronchial tree with the development of distention bronchiectasis further supports the theory of intrabronchial spread.

4. Both the gross and microscopic appearance of the metastasis closely resemble the primary benign growth in the jaw. Histologically, the section of the tumor in the lung is that of an ordinary adamantinoma.

5. There was no local lymph node involvement, and no metastases were found elsewhere in the body. This suggests some other method of spread of the secondary deposit than via the blood stream or lymphatics.

If the tumor was transplanted by aspiration it is necessary to assume that the tumor cells grew in the bronchial mucus secretion and in the mucosa. Histogenetically, adamantinoma cells arise from cells which are the precursors of enamel-forming tissue. While no similar or analogous instance of growth of tumor cells in such a tissue culture medium *in vivo* is known to the authors, it is probable that these cells are particularly hardy in their capacity to withstand adverse conditions.

SUMMARY

An instance of adamantinoma of the jaw with metastases to the lung is reported. The bronchi of the lower lobe were markedly dilated and their lumina filled with a cast of the tumor tissue. In places the parenchyma of the lung was invaded. It is suggested that the tumor tissue was aspirated into the lung from the primary tumor, via the trachea and bronchial tree, and grew primarily within the lumina of the bronchi. No similar metastatic lesion of an adamantinoma in the lung was found reported in the literature.

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Other portions of lung where the tumor fills the bronchus and invades the surrounding tissue show that the tumor within the lumen of the bronchus is identical with that described above. In addition there are small areas of hemorrhage and necrosis. In other areas tumor is seen in the surrounding pulmonary tissue, invading the wall of the bronchus from within and encroaching on the cartilage within the bronchial wall. At the points where the tumor tissue invades the cartilage the tumor cells maintain their original adamantinomatous structure.

The nodule in the left lower lobe is composed of a very necrotic hyalinized material surrounded by cellular granulation tissue which is well vascularized. No evidence of tumor is present in the necrotic nodule. The surrounding lung tissue is congested and in places atelectatic and fibrotic.

The spleen shows evidence of marked congestion. The follicles are small, arterioles thickened. There are many cellular elements in the sinuses, some of which are plasma cells and round cells. An occasional erythrophagocyte is observed.

DISCUSSION

From the review of the literature it is apparent that adamantinomas rarely metastasize. The presence of a large, secondary focus in the lung showing the histological structure of an apparently benign adamantinoma, in the instance reported in this communication is, therefore, unusually interesting.

The peculiar structure of the tumor in the lung was striking. The tumor filled the bronchial tree of the right lower lobe in a cast-like form and markedly distended the lumina of the bronchi. The bronchiectasis resulting from the inner pressure was extreme. The parenchyma, in places, and the pleura were extensively invaded. In view of this unusual manner of growth of the tumor it is suggested that tumor cells were aspirated from the primary focus in the jaw. In favor of this hypothesis are the following facts.

1. Such aspiration of tumor tissue was possible since the primary tumor in the upper jaw extended into the antrum and impinged on the nasopharynx. Furthermore, the patient had undergone several operations in which a general anesthetic had been administered.

2. The secondary tumor in the lung was present only in the right lower lobe. Jackson ³⁰ found that aspirated foreign bodies lie most frequently in this site.

DESCRIPTION OF PLATES

PLATE 81

FIG. 1. Tumor cast in a bronchus. $\times 30$.

FIG. 2. Section through tumor cast showing the histological appearance of adamantinoma. $\times 120$.

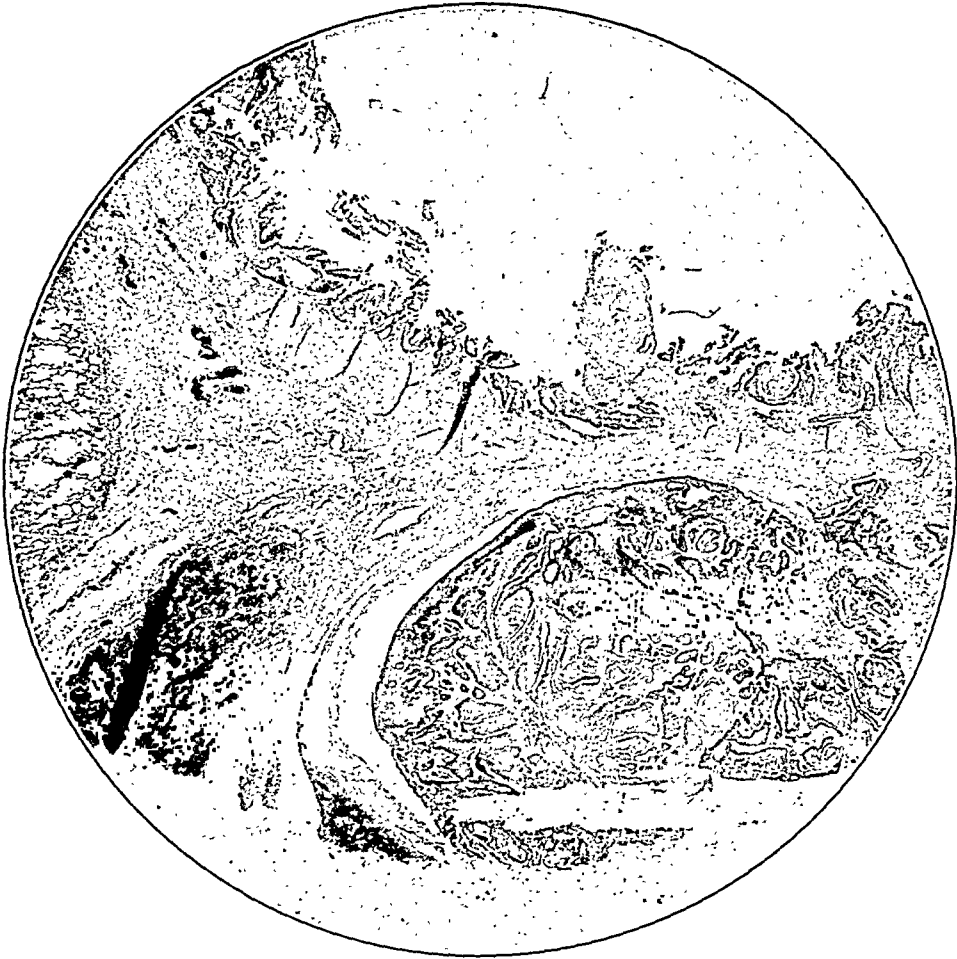
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PLATE 82

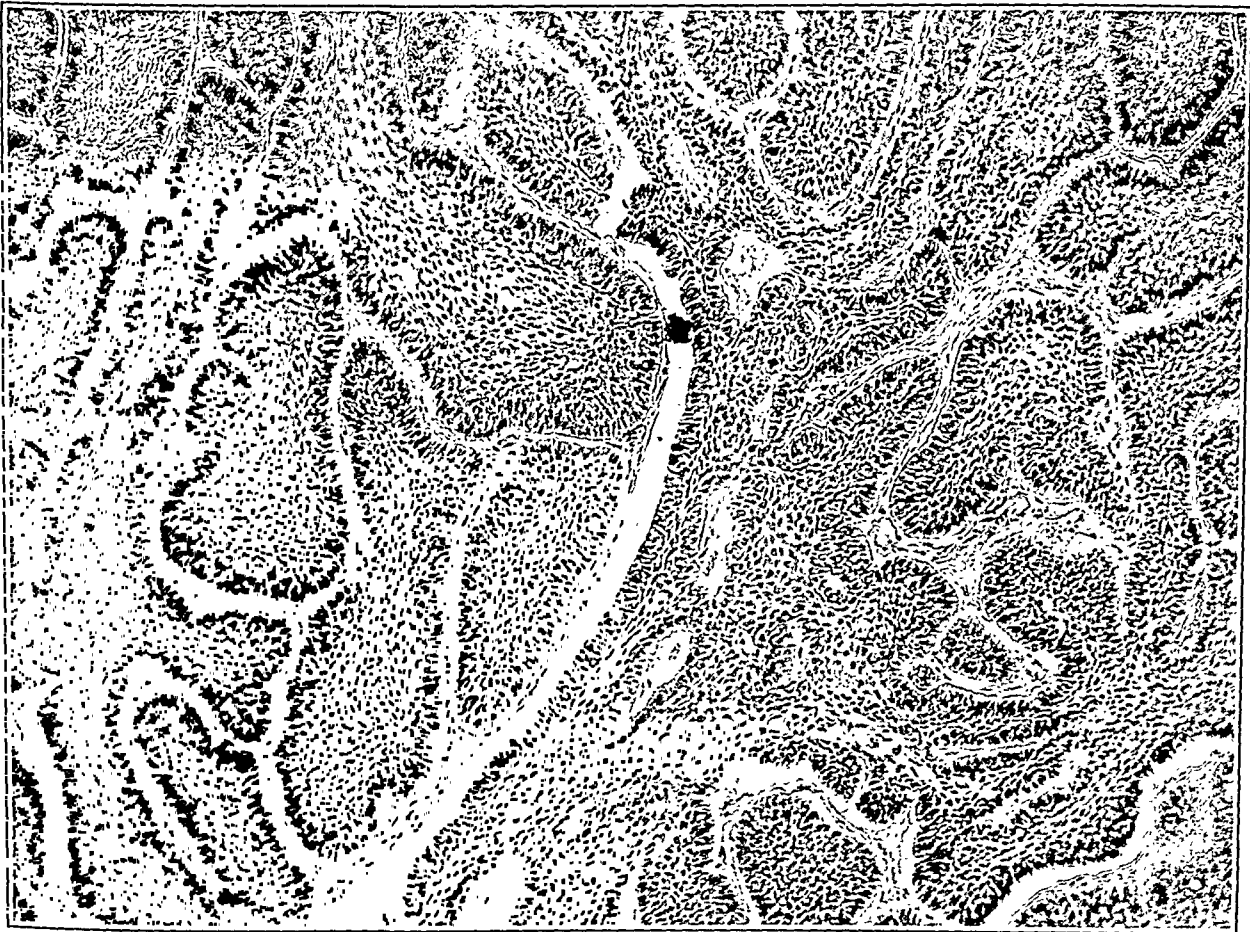
FIG. 3. Same as Fig. 2. Higher magnification. $\times 440$.

FIG. 4. Adamantinomatous tissue invading the cartilage of a bronchus.

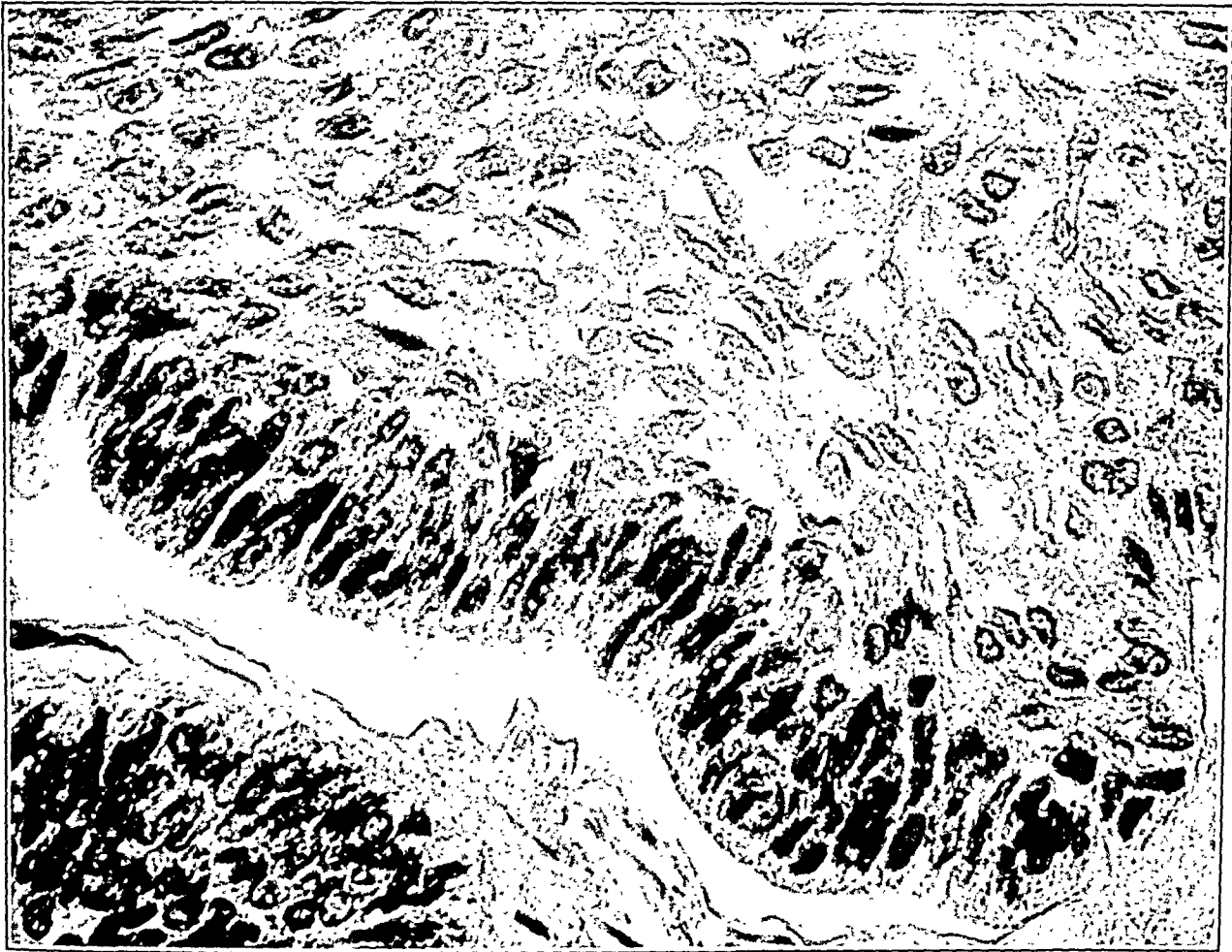
FIG. 5. Nodule in left lung showing hyaline degeneration with connective tissue capsule. $\times 30$.



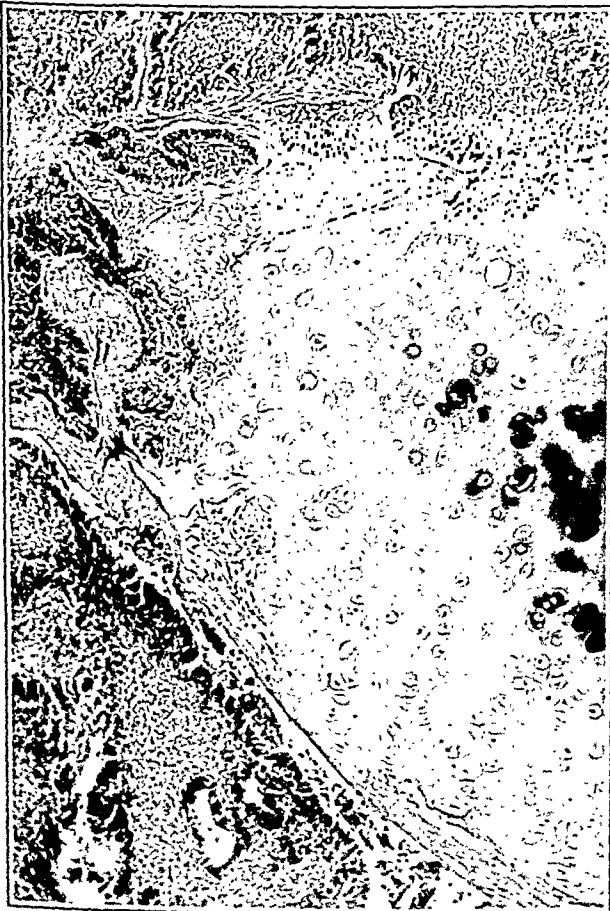
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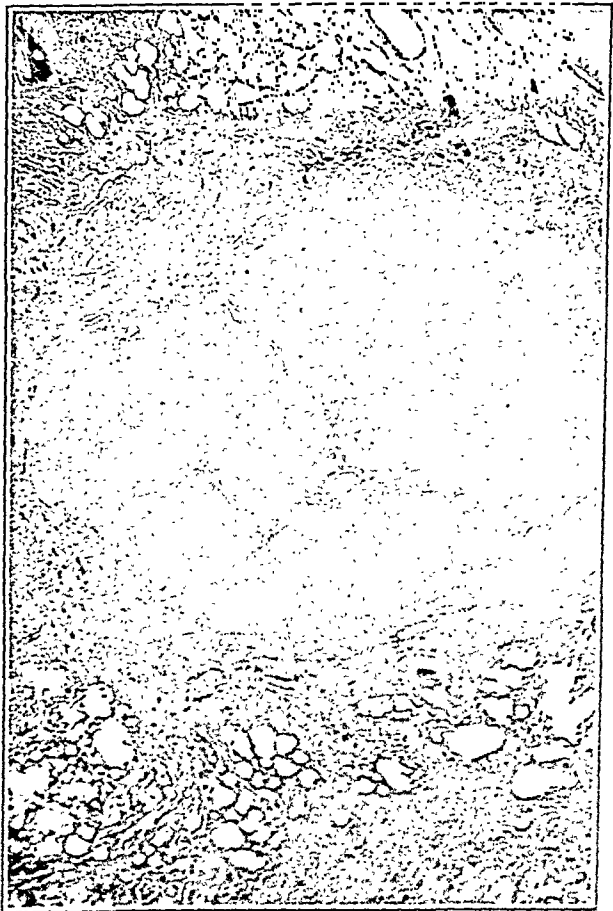
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layer of connective tissue of the intima in children as a type of hyperplastic thickening.

More definite advance in our knowledge of arteriosclerosis will be made, in the opinion of Klotz, by a careful analysis of its very early stages, particularly those occurring in childhood. Indeed, by the twentieth year of life the picture generally is too complex to allow a proper appreciation of the real factors concerned in the pathology of this disease. Upon his suggestion I investigated the aortas of twenty children between the ages of 2 days and 10 years, having in mind the pathological changes previously reported in infectious diseases (Klotz,⁸ Stumpf,⁹ and Klotz and Manning¹⁰). The material was obtained, by the courtesy of Dr. I. H. Erb, from recent autopsies at the Hospital for Sick Children, Toronto, Canada. As the work progressed, it was found that developmental changes were the most interesting feature in such material, since there is so little about them in the literature, and since they were so striking in the group of children I examined.

METHODS

In most cases the whole aorta was available. The material was fixed in 5 per cent formalin. The vessel was carefully handled when in the fresh state and was never washed in water or injured by rough usage. Five specimens from each case were examined. Portions were taken from the ascending aorta near the aortic valves, from the transverse aorta near the origin of the innominate artery, from the thoracic aorta between the fourth and fifth pair of intercostal arteries, and from the abdominal aorta above the origin of the common iliacs. As a rule the aorta was cut longitudinally before being fixed. In a few cases, the entire aorta was fixed before opening. This was done in order to avoid artefacts resulting from shrinkage, with consequent apparent thickening of the intima. In other cases, portions of the aorta were submitted to a considerable over-distention while being fixed, the ends of the vessel being tied to a lightly bent rubber tube, which was distended and maintained in a straight position by attaching it to a glass rod, and the whole was then immersed in formalin. The anterior and posterior parts of the vessel were always carefully determined before removing blocks for section. Complete transverse sections were obtained in most cases.

Frozen sections were employed, and the following stains were used on each specimen: Schultz' stain for demonstrating the mucoid

TRANSIENT PACHYMEMIA OF THE INTIMA OF THE AORTA WITH REFERENCE TO JUVENILE ARTERIOSCLEROSIS *

C. MAGARINOS TORRES, M.D.

*(From the Department of Pathology and Bacteriology, University of Toronto,
Toronto, Canada)*

During recent years much interest has developed in the subject of juvenile arteriosclerosis, and, as a result of careful observation and study, the conclusion that arteriosclerosis occurs in children much more frequently than was formerly believed is being commonly accepted. Although the names of many different authors who have dealt more or less directly with this matter are mentioned by Zeek¹ in a recent review of the literature, little is to be found on the subject beyond the fundamental contributions of Thoma and those of Jores concerning developmental changes in the intima of the aorta after birth. Such changes evidently occur, for the majority of authors maintain that the intima in the newborn is formed by endothelium and the elastica interna alone. The excessive development of the subendothelial layer of the intima, however, can hardly be separated from the early arteriosclerotic changes in young subjects.

Thoma² has shown that the connective tissue layer of the intima develops after birth, and is a rather sparse structure below the level of the ductus arteriosus. Gradually, in the course of the first year, it begins to increase, and at 5 years a definite connective tissue layer is present in the descending and abdominal aorta. This occurs only in the portions of the vessel in which the so-called "Nabelblutbahn" previously existed. Thoma^{3,4} states that the cessation of the placental circulation is responsible for a physiological hyperplasia of connective tissue in the intima. This hyperplasia results as a compensatory process and is referred to by him as "kompensatorische Intimawucherung." Thoma's⁵ view, explaining the thickening of the intima by connective tissue as a result of a slowing of the blood stream, has not been universally accepted. Jores^{6,7} considers the

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sections in a concentrated solution of Sudan III in 70 per cent alcohol. (2) Dip in 70 per cent alcohol. (3) Wash in water. (4) Stain in 5 per cent aqueous solution of cresyl violet for 3 minutes. (5) Wash in water. (6) Stain in dilute Harris' hematoxylin for 1 to 3 minutes. (7) Wash in water. (8) Mount in levulose jelly (8 gm. levulose in 10 cc. water).

OBSERVATIONS

CASE 259. Baby T., male, 2 days old. Autopsy $2\frac{1}{2}$ hours postmortem. *Anatomical diagnoses*: Intracranial hemorrhage; laceration of tentorium cerebelli.

The intima throughout the aorta fails to show a subendothelial layer. In the thoracic aorta the elastica interna is formed by very irregular, short, homogeneous, bluish-stained segments (Fig. 1a) separated by narrow, tortuous, pink-stained portions. Its internal surface shows numerous processes simulating buds or sprouts (Fig. 1a). In the media the outlines of the central elastic membranes are not straight and regular (Fig. 1b); the more external fibers are formed by wide, homogeneous, bluish segments alternating with others, pale pink or unstained ("tapeworm appearance" Fig. 1c). In the abdominal aorta, however, the laminae of the midzone are quite continuous, straight and uniformly blue.

CASE 251. R. M. G., female, 14 days old. Autopsy 4 hours postmortem. *Anatomical diagnoses*: Acute omphalitis; septicemia (*Streptococcus hemolyticus*); peritonitis; leptomeningitis.

The intima throughout the aorta is formed by endothelium and the elastica interna, the latter quite distinct in the abdominal aorta. Tested by Schultz' method the elastic membranes show a varying appearance at different levels. In the media of the ascending aorta they are formed by discontinuous, pale greenish, homogeneous segments embedded in a hyaline substance suggesting the formation of elastic fibers in an amorphous matrix. They simulate the early developmental structure, the elastic substance existing only in discontinuous plates. In the transverse aorta this appearance is less marked and is limited to the more internal or the more external elastic lamellae; the central ones are formed by continuous homogeneous strands (elastic substance) which run in parallel bands. In the thoracic aorta these features are less marked. Here, on its inner surface, the elastica interna presents numerous fine, oblique processes which stain like the elastic substance, while its external surface is regular. In the abdominal aorta the developmental characteristics are limited to the elastica interna, while the remaining elastic membranes appear uniformly greenish and show a regular arrangement in the media.

In the ascending aorta a marked perivascular infiltration by polymorphonuclear and endothelial leucocytes is noted around the vasa vasorum. Some of these inflammatory cells extend through the remaining portions of the vessel wall.

CASE 253. K., female, 6 weeks old. Autopsy $5\frac{1}{2}$ hours postmortem. *Anatomical diagnoses*: Empyema of cerebral ventricles; spina bifida; acute peritonitis; acute omphalitis.

In the ascending aorta the intima is very thin and formed by endothelium and elastica interna alone. In the transverse, thoracic and upper abdominal aorta the intima presents a continuous subendothelial layer that gradually becomes

or chromotropic substance of the connective tissue; Sudan III-cresyl violet; Sudan III-cresyl violet-hematoxylin; and Sudan III-hematoxylin. Smith's method (Nile blue sulphate) and examination under the polarising microscope with the Nichols prism were also used for the identification of the fats. In a number of instances paraffin sections were studied for comparison. They were stained by hematoxylin-eosin, Schultz' stain and Verhoeff's elastic tissue method.

Schultz' Stain for Vascular Mucous or Chromotropic Substance

Formalin fixation, paraffin or frozen sections. (1) Stain sections in 5 per cent aqueous solution of cresyl violet extra (Kresylechtviolett R extra) for 20 to 30 minutes. (2) Differentiate quickly in a very dilute aqueous solution of acetic acid (2 to 3 drops in 30 cc. of water) until no clouds of stain come from the sections. (3) Rinse in water. (4) Wash briefly in very dilute aqueous solution of ammonia. (5) Wash thoroughly in several changes of water. (6) Mount in levulose jelly.

The color will last for several weeks: nuclei violet, the chromatin appearing very well stained; vascular connective tissue in varying shades of bright purplish red to pale pink; collagen pale grayish blue or unstained; muscle tissue pale bluish; red blood corpuscles bluish or greenish; fibrin often bright blue; elastic tissue sometimes unstained in young subjects and later in varying shades of blue to bright cobalt blue. For the aorta of infants the most satisfactory procedure is to stain the frozen sections for 1 hour, immerse them briefly in dilute aqueous acetic acid and then wash them thoroughly in water. The elastic tissue stains pale greenish blue in this way.

Though non-specific, Schultz' stain is very useful for the study of the elastic tissue as well as the chromotropic substance in the aorta. It demonstrates the segments of the elastic membranes in which the specific elastic substance is already formed, while in those in which this substance is lacking, a metachromatic color reaction is obtained. Therefore, the less advanced the developmental stage of the elastic membranes are in childhood, the more they will appear as rose-red or colorless segments interposed between greenish blue and homogeneous ones.

A variant of this method is useful for showing the flocculation of the chromotropic material as well as fatty changes. (1) Stain frozen

terna is formed by homogeneous, bluish segments (Schultz' stain) separated from one another by narrow, tortuous, pink or colorless portions; numerous delicate processes were also seen on its inner surface.

CASE 1. D. R., male, $3\frac{1}{2}$ months old. Autopsy 9 hours postmortem. *Anatomical diagnoses*: Nasopharyngitis; otitis media.

In the ascending aorta the intima presents no subendothelial layer. The latter appears continuous at the thoracic and lower abdominal aorta. At the upper abdominal aorta it is discontinuous, presenting a gradual thickening at the back part of the vessel, and forming a typical sloping overgrowth. The structure shows star-shaped fibroblasts resembling young connective tissue. Fatty degeneration of the lining endothelial cells and of fusiform cells in the subendothelial layer is found in the thoracic and abdominal aorta, being distinctly more marked at the sloping overgrowth.

CASE 265. C. B., female, aged 4 months. Autopsy 19 hours postmortem. *Anatomical diagnoses*: Acute enteritis, nasopharyngitis.

The subendothelial layer is lacking in the ascending and transverse aorta, existing and continuous at the thoracic aorta, and discontinuous in the abdominal aorta. The thickness of the intima is not uniform, being thin in the thoracic aorta and terminating in tapering ends at the abdominal aorta. Sloping overgrowths are present in those portions. Fatty changes are quite marked in the intima of the thoracic aorta. Cellular infiltration of the media and around the vasa vasorum is noted at the ascending aorta. The elastica interna presents a discontinuous appearance.

CASE 261. R. A., male, aged 5 months. Autopsy 14 hours postmortem. *Anatomical diagnosis*: Acute purulent leptomeningitis.

In the transverse aorta the intima does not show a subendothelial layer, while a sloping overgrowth is found in the thoracic portion. The latter appears thick and bulges a little above the normal level of the intima (Fig. 6 a). The thickness of the intima in this portion corresponds to a little less than half that of the media. The structure shows flattened cells, stellate or spherical in outline, many of them containing fat droplets and forming distinct fibrous lamellae. Less marked sloping overgrowths are noted at the abdominal aorta (Fig. 6 b and c); the subendothelial layer terminates in tapering ends in the specimen near the common iliaes.

Marked fatty degeneration and flocculation of the chromotropic tissue are seen in the intima at the sloping overgrowths, especially in the thoracic aorta. They do not coexist, however, in the same strata, as the fatty degeneration is more marked near and on the endothelium, while the mucoid degeneration is more intense in the deeper strata adjoining the elastica interna. Examination under the polariscope with the Nichols prism reveals anisotropic fat droplets.

Comment: A sloping overgrowth was definitely more marked at the thoracic than at the abdominal aorta, and more in the lower than in the upper abdominal aorta.

The study of sections from the thoracic aorta (Schultz' stain) suggests that the development is more advanced in the elastic membranes in the middle portion of the media than at the internal or external portions (Fig. 7).

It is curious to observe in this case the presence of pathological changes just at the developmental thickenings. This is true especially with regard to fatty changes; the more marked the thickening the more numerous are the cells containing fat droplets.

thicker and forms sloping overgrowths. These are more marked at the transverse and upper abdominal portions than at the thoracic, and consist of fibrous connective tissue enclosing flattened and star-shaped fibroblasts as well as smooth muscle fibers. Large vacuolated cells, some of them containing fat droplets and presenting vacuolar degeneration and necrosis, are also demonstrable. In the abdominal aorta near the common iliacs a very thin subendothelial layer exists.

Fatty degeneration of the elastica interna is found in the abdominal aorta where this membrane is very distinct. Slight flocculation or mucoid degeneration of the chromotropic tissue, as well as perivascular infiltration around the vasa vasorum, are present in the ascending and transverse aorta. While in the transverse aorta almost all the elastic membranes possess discontinuous, greenish-stained segments (Schultz' stain), this aspect is less marked in the thoracic aorta and still less in the abdominal aorta. In the latter portions this discontinuity concerns chiefly the elastica interna.

Comment: In this case sloping overgrowths of the intima are found not only in the thoracic and abdominal aorta, but also in the transverse aorta as well. They are less marked in the thoracic segment than in the other parts. Definite degenerative changes in the intima represent a response to the infectious intoxications that existed practically from birth. This case illustrates well the difficulty of distinguishing between developmental and pathological changes in the aorta of infants. The irregular arrangement of the mononuclear and connective tissue cells, as well as the presence of intracellular and free fat, suggest a definite pathological change.

CASE 263. M. O., male, aged 7 weeks. Autopsy $2\frac{1}{2}$ hours postmortem. *Anatomical diagnoses:* Marasmus; congestion of the abdominal viscera.

The subendothelial layer is found only in the abdominal aorta; it is lacking completely in the other segments. It is continuous and forms a quite marked sloping overgrowth 3 mm. above the origin of the common iliacs, being discontinuous just above the celiac axis (Fig. 2). A few cells containing fat droplets exist at the sloping overgrowth.

CASE 246. W. M. D., male, 2 months old. Autopsy $20\frac{1}{4}$ hours postmortem. *Anatomical diagnoses:* Otitis media; edema of brain.

The subendothelial fibrous layer of the intima is found only in the lower abdominal aorta. It is discontinuous, forming three different, rather small, sloping overgrowths, when a complete transverse section is examined. Slight perivascular infiltration around the vasa vasorum at the ascending aorta is the only pathological finding.

CASE 254. M. E., female, $2\frac{1}{2}$ months old. *Anatomical diagnosis:* Bronchopneumonia (both lungs).

The subendothelial fibrous layer of the intima is continuous at the transverse, the thoracic and the abdominal aorta near the origin of the common iliacs, but discontinuous at the mouth of the left renal artery. At the posterior part of the vessel a very marked thickening of the subendothelial layer is found in the transverse, thoracic and abdominal aorta (Figs. 3, 4 and 5). The structure consists of young connective tissue, except in the transverse aorta. Fibroblasts containing anisotropic fat droplets, vacuolation of the connective substance, and fine extracellular fat droplets near the elastica interna are found in the sloping overgrowths in the abdominal aorta. The degenerative processes are slight and not found in other portions, being clearly limited to the sloping overgrowths. Chromotropic tissue is more evident in the inner half of the media. The elastica in-

and less cellular than in the thoracic aorta. Such peculiarities in the normal development of the intima are confusing, as they suggest pathological changes.

CASE 264. D. S., male, aged 7 months. Autopsy, $1\frac{1}{4}$ hours postmortem. *Anatomical diagnoses:* Leptomeningitis; left otitis media (pneumococcus).

There was a well marked thickening of the subendothelial layer of the intima in the ascending and transverse portions of the aorta, while the thoracic aorta showed more diffuse thickening in the same layer. Occasionally small deposits of fat were also found in the connective tissue cells. The subendothelial structure formed separate bands in the abdominal aorta and shaded off to very thin structures in its lower portion.

Comment: It is interesting that although this child had suffered from an acute infectious process, the subendothelial layer of the aorta is distinctly less developed than in most of the children of this group. It would appear that these changes in the intima represent different stages in the development of the subendothelial layer.

CASE 255. M. L., female, aged $7\frac{1}{2}$ months. Autopsy 12 hours postmortem. *Anatomical diagnoses:* Otitis media; sinus thrombosis (superior longitudinal sinus).

The continuous layer of subendothelial fibrous tissue rich in elastic fibers is present in the thoracic and abdominal aorta, while some fatty changes are localized in the ascending limb and the lower abdominal aorta.

Comment: Sloping overgrowths of the intima are absent in this infant of $7\frac{1}{2}$ months, although they were quite marked in children of 4, 6 and 10 years of age.

CASE 244. T. M. R., female, aged 18 months. Autopsy 1 hour postmortem. *Anatomical diagnosis:* Diphtheria.

The subendothelial layer is undeveloped in the ascending and transverse aorta, but appears well formed in the thoracic aorta, forming a sloping overgrowth. In the upper abdominal aorta the subendothelial layer is disposed uniformly, while sloping overgrowth is again demonstrable near the origin of the common iliacs. Slight cellular infiltration around the vasa vasorum at the ascending and transverse aorta is the only pathological change of note.

CASE 248. M. G., female, aged 21 months. Autopsy 15 hours postmortem. *Anatomical diagnoses:* Lobar pneumonia; acute fibrinous pleurisy; otitis media.

In the thoracic and abdominal aorta the subendothelial layer is discontinuous, forming a typical, quite marked, sloping overgrowth near the mouth of the celiac axis (Fig. 8). The structure shows abundant connective tissue cells, some of them flattened and disposed in fibrous lamellae. A slight thickening of the intima is also seen in the lower abdominal aorta, above the origin of the common iliacs. No sloping overgrowth is found at the thoracic aorta. The elastica interna is formed by greenish segments (Schultz' stain), which alternate with pink or colorless portions.

CASE 252. R. M., male, aged 3 years. Autopsy 18 hours postmortem. *Anatomical diagnosis:* Diphtheria.

In this case of diphtheria, a slight perivascular infiltration around the vasa vasorum in the ascending aorta is almost the only pathological change demonstrable. A continuous subendothelial layer in the intima is found at the thoracic and abdominal aorta, while it is thin or lacking at the ascending aorta, and does not exist at the transverse aorta. A typical sloping overgrowth is detected only in the abdominal aorta near the common iliacs. One gains the impression that a sloping overgrowth is actually disappearing in the thoracic aorta, while another is demonstrable in the abdominal aorta near the bifurcation.

CASE 260. Y. F., female, aged 5 months. Autopsy 12 hours postmortem. *Anatomical diagnoses*: Septicemia; infected wounds of the skin; otitis media (*Streptococcus hemolyticus*).

A discontinuous subendothelial layer of connective tissue is found in the ascending and transverse aorta. In the remaining portions of the aorta this layer is continuous throughout the intima, forming a marked overgrowth in the thoracic aorta.

A few cells containing fat droplets and large vacuoles are seen in the intima, especially in the overgrowth of the thoracic aorta. Moreover, in that portion peculiar homogeneous, non-cellular, round masses, staining grayish blue with cresyl violet are lying among collagen fibrils. Marked flocculation of the chromotropic tissue is seen in the internal portions of the media of the transverse aorta.

CASE 240. K. H., female, aged 7 months. Autopsy 14 hours postmortem. *Anatomical diagnoses*: Acute bronchitis; double otitis media.

In the ascending aorta the intima is formed by the endothelium and elastic layer. The intima of the transverse aorta presents a well developed layer in which fibroblasts may be seen, while the elastica interna is distinguished with difficulty. In the thoracic aorta, the elastica interna is very distinct. The intima becomes much thickened at one point and then diminishes, so that a typical sloping overgrowth may be seen. It is formed by endothelium, a subendothelial or connective tissue layer and an elastic layer. In the latter, many smooth muscle fibers exist. The subendothelial layer contains branched connective tissue cells, more or less regularly arranged. The developmental and fatty changes simulate arteriosclerotic changes. In the abdominal aorta the intima is thinner, presenting a continuous subendothelial layer. No sloping overgrowth is formed in spite of a varying thickness of this layer.

Chromotropic connective tissue is more distinct in the internal half of the media. The elastica interna presents a very irregular inner surface. In the thoracic aorta it is formed of short, homogeneous segments, which probably correspond to islands of the elastic substance. They alternate with narrow, immature portions. The same appearance is found in the abdominal aorta, but the elastic segments are longer. The elastic lamellae of the media stain uniformly greenish blue, except some external ones, in which greenish segments alternate with pink ones.

In transverse paraffin sections stained by Verhoeff's method, the elastica interna is formed of small, irregular, deeply stained fragments. As these short fragments are quite distinct and separated from one another by narrow, colorless spaces, a rough and irregular surface is produced. Thoma² has described this appearance in the newborn as "ein eigenartige körniges Gefüge entsprechend einer Zusammensetzung aus dicht gedrängten, longitudinalen Faserbündeln und Fasernetzen."

Comment: A more or less advanced stage of arteriosclerosis is simulated by a developmental connective tissue plaque in the thoracic aorta of a child of 7 months, dying from an infectious process. Fatty changes are present in the overgrowth of the thoracic aorta. The fat is contained within cells, apparently fibroblasts, and appears to be of the nature of a cholesterol ester.

The intima of the abdominal aorta is of uniform thickness and presents a continuous subendothelial layer. If a sloping overgrowth has previously existed in this portion, it has now completely disappeared and the coat is distinctly thinner

In the abdominal aorta, the intima becomes progressively thicker, measuring 175 microns near the celiac axis. A typical sloping overgrowth is formed, similar to that found in Cases 241, 261 (Fig. 6), and 254 (Figs. 3, 4 and 5) children respectively 4 years, 5 months and $2\frac{1}{2}$ months old. The structure shows numerous adult fibroblasts in regular arrangement.

Comment: The intima presents a continuous subendothelial layer throughout the vessel. This coat is thin and of uniform thickness in the ascending, transverse and thoracic aorta. While such distribution indicates a fairly advanced stage in the development of the intima, typical sloping overgrowths with all the characteristics described in Cases 254, 261 and 241, may be seen in the abdominal aorta. No fatty changes are noted; flocculation or mucous degeneration in the ascending aorta is the only pathological finding.

CASE 245. D. O., male, aged 10 years. Autopsy 3 hours postmortem. *Anatomical diagnoses:* Cerebral abscess (left hemisphere); acute purulent leptomeningitis; otitis media.

In the ascending aorta the intima is formed by endothelium and elastica interna only, the latter not being easy to distinguish from the other elastic membranes. In the transverse aorta a few stellate cells lying in a thin network of elastic fibrils are present. In the thoracic and abdominal aorta the intima shows a very distinct elastica interna and a continuous subendothelial layer. Its thickness is more or less uniform and distinctly thinner than in the aorta of infants 2 to 4 months old. Near the celiac axis, however, the intima very gradually becomes thicker, so that a cellular overgrowth is produced. Fatty changes appear focally at the mouths of the intercostal arteries. A small amount of intra- and extracellular fat is found in this limited portion of the intima. Mucous degeneration was very marked in the chromotropic tissue throughout the media in the ascending and transverse aorta.

Comment: Only a slight sloping overgrowth is detected in the upper abdominal aorta. The intima is distinctly in an advanced stage of development in the thoracic and abdominal aorta, as compared with that in other children of this group. It is continuous, thin, fairly uniform in thickness, with fibroblasts disposed evenly and in collagenous lamellae. In the ascending and transverse aorta, however, no subendothelial layer exists. The infectious disease is probably responsible for the superficial fatty streaks at the mouths of the intercostal arteries and for the marked flocculation (mucous degeneration) of the chromotropic connective tissue in the ascending and transverse aorta.

DISCUSSION

Thickenings of the intima on the posterior part of the aorta are demonstrable with great constancy in specimens from a group of children of different ages. They correspond, in some respects, to what Thoma⁴ has described as "kompensatorische Intimawucherung," but appear to represent only the normal development of the intima, and in particular, of its subendothelial layer. As a matter of fact, the developmental plaques are not seen by the naked eye, as they do not rise above the surface of the aorta. This feature is important, as it distinguishes them from the common pathological lesions.

This case illustrates well the way in which the maturation or development of the elastic membranes proceeds. In the ascending and transverse aorta the elastica interna is not easily distinguished from the more internal elastic membranes of the media, and all of them present a segmented appearance (Schultz' stain), while in the thoracic and abdominal portions it is quite distinct.

CASE 241. P. C., male, aged 4 years. Autopsy $7\frac{1}{2}$ hours postmortem. *Anatomical diagnoses*: Osteomyelitis (left ilium); septicemia (*Staphylococcus aureus*).

The intima is very thin and formed only by endothelium and elastica interna from the ascending to the abdominal aorta. In the lower abdominal aorta immediately below the superior mesenteric, a discontinuous subendothelial layer is seen, which forms a typical sloping overgrowth at the back part of the aorta. A similar sloping overgrowth always posteriorly may be traced from the mouth of the right renal artery downward to a point 11 mm. above the origin of the common iliacs. The sloping overgrowth appears more marked as we trace downward and approach the origin of the common iliacs. The structure of the dorsal connective tissue plaque shows endothelium, young fibroblasts in a broad layer and some smooth muscle fibers near the elastica interna.

Islands of elastic substance in the elastica interna are seen clearly in the thoracic aorta (Schultz' stain), while the segments in which no elastic substance exists show a metachromatic reaction. Small pedunculated processes give an indented appearance to the internal surface of the elastica interna.

Comment: A dorsal connective tissue plaque limited to the abdominal aorta is continuous and can be followed upward from the origin of the common iliacs to the superior mesenteric artery, covering a distance of about 29 mm. of the aorta and always located at its posterior border. The subendothelial layer is no longer found above the orifice of the celiac axis. The dorsal connective tissue plaque, being quite marked in the lower abdominal aorta, becomes gradually attenuated and disappears entirely at the upper abdominal aorta.

CASE 262. M. T., female, aged 5 years. Autopsy 6 hours postmortem. *Anatomical diagnosis*: Acute fibrinopurulent peritonitis.

The subendothelial layer of the intima is well developed, continuous, and formed by flattened fibroblasts disposed in definite lamellae in the thoracic and abdominal aorta (Fig. 9). It has a more or less uniform thickness, no sloping overgrowth being detected in any portion. In the ascending and transverse aorta no subendothelial layer is found.

Comment: The absence of sloping overgrowths and the structure of the continuous subendothelial layer at the thoracic and abdominal aorta suggest a further stage in the development of the intima. Anisotropic fat droplets inside the lining endothelium and fibroblasts of the subendothelial layer, as well as extracellular fat droplets, are detected in the ascending and the abdominal aorta.

CASE 238. S. T., female, aged 6 years. Autopsy $12\frac{1}{2}$ hours postmortem. *Anatomical diagnosis*: Poliomyelitis.

In the ascending aorta the intima presents a fairly developed subendothelial and elastic layer. There are no fatty changes. Chromotropic connective tissue (Schultz' stain) is uniformly developed in the media, slightly increased in amount and flocculent in places. In the transverse aorta the intima shows a subendothelial and elastic layer with regularly arranged fibroblasts in the interspaces of elastic and collagen fibers. The elastica interna presents much longer blue-stained segments than those seen in younger infants. In the thoracic aorta the subendothelial layer is well developed, containing smooth muscle fibers in its deeper portion. The intima is of more or less uniform thickness, and rather thin.

even in very young subjects (Case 253). Such thickened portions, however, have a varied distribution along the aorta (Table I and Text-Fig. 1). They do not represent, therefore, a regular and more advanced growth of the intima, neither are they more marked in a particular segment of the aorta. In Case 253, an infant 6 weeks old, a great thickening is found in the upper abdominal aorta (Table I), while this is not observed in Case 246, an infant 2 months old. It is

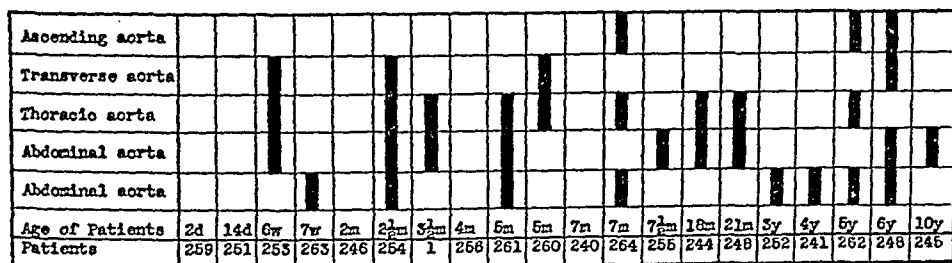
TABLE I

Thickness in Microns of the Intima of the Aorta in a Group of Children between the Ages of 2 Days and 10 Years

Patient	Age	Sex	Ascending aorta	Transverse aorta	Thoracic aorta	Upper abdominal aorta	Lower abdominal aorta
259	2 d.	M					
251	14 d.	F					
253	6 w.	F		60-160	80-120	90-210	
263	7 w.	M			40	40-90	20-160
246	2 m.	M				35	25-70
254	2½ m.	F		130-255	60-220	40-135-250	20-70-290
1	3½ m.	M	65		60-110	25-105	35-75
265	4 m.	F			30-70	35	60
261	5 m.	M			65-245	80-110	40-75-225
260	5 m.	F	85	40-85-210	50-200	35-85	35-65
240	7 m.	F	40		50	50	30
264	7 m.	M	115		50-100	40-50	40-180
255	7½ m.	F			40-90	15-130	40-100-140
244	18 m.	F	25	50	95-210	75-100	35
248	21 m.	F			65-170	165	20-75
252	3 y.	M	20-40		30-60	40-60	45-110
241	4 y.	M				25-60	30-40-115
262	5 y.	F	180		65-110	50-80	60-110
238	6 y.	F	80-100	110	90	30-175	35-190
245	10 y.	M			70	35-150	35-65

Not less important is the microscopic aspect. Such developmental plaques, when complete transverse sections are examined, increase gradually in thickness on the inner side of the elastica interna (Figs. 2, 3 and 8), until a maximum is reached. Beyond this, they again become gradually attenuated. Sometimes the subendothelial layer disappears completely at the edges, so that the sloping overgrowth appears more marked. This justifies the term "sloping overgrowth" of the intima so often used in this paper. The thickening is essentially produced by the subendothelial layer which appears very cellular.

It would be well to point out that the thickness of the intima in this group of children (Table I) is often much greater than that commonly reported as normal. My measurements gave from 10 to 15 microns in transverse frozen sections mounted in gelatin. Langhaas,¹¹ for example, gives 0.015 mm. to 0.025 mm. as the normal for three children, 4 days, 1½ years and 10 years old respectively, and Thoma² 30, 35 and 41 microns for a child 7 weeks old. Text-Fig. 1



TEXT-FIGURE 1

Graphic representation of Table I showing the distribution of thickness over 100 microns

is a graphic representation based on Table I of the distribution along the aorta of portions in which the thickness of the intima is over 100 microns. From this figure it may be seen how wide the variations are, either in the same subject or in a group of children of different ages.

These data do not agree with the view generally accepted that, if in the newborn the intima is very thin, it increases in thickness gradually and regularly with age, presenting a more or less constant thickness for each age. On the contrary, it seems that during childhood, as the subject is actively growing, the intima presents a variable thickness, which is often considerable and exceeds 200 microns

TABLE II

Thickness in Microns of the Tunica Media of the Aorta in a Group of Children between the Ages of 2 Days and 10 Years

Patient	Age	Sex	Ascending aorta	Transverse aorta	Thoracic aorta	Upper abdominal aorta	Lower abdominal aorta
259	2 d.	M	890	725	625	560	400
251	14 d.	F	875	750	590	500	250
253	6 w.	F		580	650	610	350-450
263	7 w.	M	650-1080	790	500	500	460-550
246	2 m.	M	810	800	650	410-650	460
254	2½ m.	F	1470	1540	760	550-660-820	450-530
1	3½ m.	M	680		580	360	380
265	4 m.	F	950	950	430-540	490	570
261	5 m.	M		910	490-560	500-540	400
260	5 m.	F	940	600	600	500	380
240	7 m.	F	1000	800-980	640	480	420
264	7 m.	M	560-840	800-920	470-560	420	350-370
255	7½ m.	F	1000	920	650	590	520
244	18 m.	F	810	750	505	590	450
248	21 m.	F			480	600	480
252	3 y.	M	900	750	570	500	450-600
241	4 y.	M	800-1110	1140	760	620	600
262	5 y.	F	1450	770	560	490	480
238	6 y.	F	1040	1000	640	550	600
245	10 y.	M	700	1550-1800-1900	670	750	630

Why in childhood does a transient thickening develop in the intima of the aorta? In Table III are tabulated the circumferences of the thoracic and abdominal aorta as measured in some of the specimens preserved in formalin. In order to afford, as far as possible, a comparative study of such data in a group of children of different ages, in every case identical portions were selected, namely the mouths of the fourth pair of intercostal arteries and of the celiac axis.

present in children 6 weeks old, $2\frac{1}{2}$, $3\frac{1}{2}$, 5, 7, $7\frac{1}{2}$, 18 and 21 months old, and 3, 4, 5, 6 and 10 years old (Table I), either in the transverse, thoracic and upper and lower abdominal aorta at the same time (Case 254); at the transverse, thoracic and upper abdominal aorta (Case 253); at the thoracic, and upper and lower abdominal aorta (Case 261); at the transverse and thoracic aorta (Case 260); at the ascending, thoracic and lower abdominal aorta (Cases 264 and 262); at the upper and lower abdominal aorta (Case 255); or only at the upper (Case 245), or lower abdominal aorta (Cases 263, 252 and 241). On the other hand it is lacking in infants 2, 4 and 7 months old (Cases 246, 265 and 240 — Table I and Text-Fig. 1).

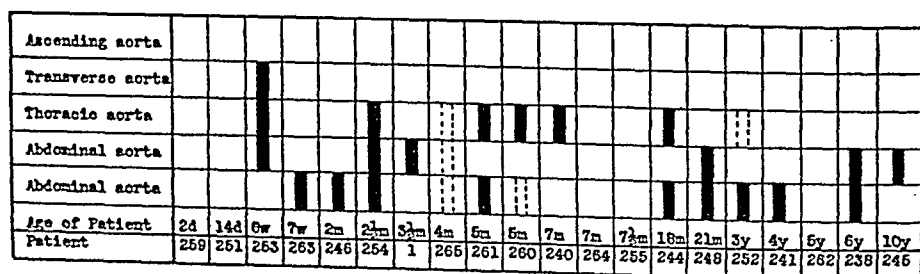
One gains the impression that these thickenings, instead of being permanent and representing in a particular subject the normal thickness of the intima at such an age, are in fact transient. They tend to disappear soon after being formed and as the child grows up, reappearing, however, later on in the same individual when the conditions giving rise to them are again met with. This, in all probability, would be reproduced several times during infancy in the same subject.

This assumption is corroborated in the study of the location and the structure of the sloping overgrowths in children of this group. Well marked and cellular sloping overgrowths, indicating a process which is actually going on, appear in Cases 254, 261, 241 and 238, children respectively $2\frac{1}{2}$ months, 5 months, 4 years and 6 years old, presenting a similar structure in all of them. This suggests a periodic recurrence of developmental thickenings in childhood. Their absence, or at least their non-existence as structures with well defined microscopic features, in Cases 264, 255 and 262, children respectively 7 months, $7\frac{1}{2}$ months and 5 years old, suggests that they may be transient rather than permanent morphological features in the aorta of infants.

In some cases sloping overgrowths are found in more than one portion of the aorta at the same time, and are lacking in intermediate portions. This suggests different and independent stages of a peculiar phenomenon occurring synchronously at different places in the same aorta. Again indefinite sloping overgrowths are noticed in other cases, as if the intermediary stages of such thickenings were just appearing or disappearing. This is in accordance with the idea of transient and recurrent morphological changes in infancy.

Evidence is therefore available, suggesting that the growth of the aorta does not proceed in a regular and uniform way throughout the vessel. Sometimes the abdominal aorta increases more rapidly than the thoracic. At other times the reverse takes place, but a constant rate is never maintained. This phenomenon also would take place during the active growth of the subject, and probably is reproduced several times in the same individual.

With regard to this view one must consider, furthermore, the close relation between the growth of the aorta and that of the bony structures. It becomes obvious that developmental changes will be more marked in the descending and abdominal than in the ascending and transverse aorta. It is curious to note that Text-Fig. 3, which is a graphic representation of the distribution of sloping overgrowths in the different portions of the aorta, as they appear in this group of children, agrees in a general way with Text-Fig. 1, which represents the distribution of thicknesses over 100 microns. The



TEXT-FIGURE 3

Graphic representation of the distribution of sloping overgrowth of the intima in the aorta of a group of children of different ages

two findings correspond and may be explained as developmental but transient thickenings of the subendothelial layer taking place periodically in several and different portions of the aorta.

This may occur: (a) when the growth is more rapid in the subendothelial layer of the vessel than in the vessel as a whole; (b) when the rate of growth between two adjoining portions of the aorta is altered so that one grows more rapidly than the other. In both cases a thickening of the subendothelial layer will be produced which is, however, transient and discontinuous in the length of the vessel and tends to be reabsorbed as the rate of growth changes at that point.

No normal children, of course, were examined. Most of them were undernourished infants, often with a history of gastro-intestinal

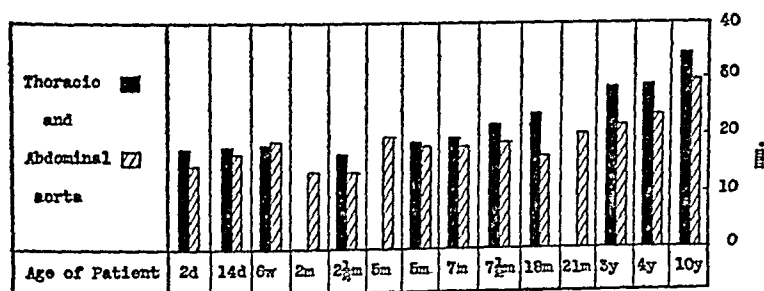
Curious variations are demonstrable in the rate of growth of the circumferences of the thoracic and upper abdominal aorta, as seen in Text-Fig. 2, which is a graphic representation of Table III.

TABLE III

Circumference in mm. of the Thoracic and Upper Abdominal Aorta in a Group of Children between the Ages of 2 Days and 10 Years

Thoracic aorta	17	17.5	18		16.5		18.5	19.5	21.5	23.5		28	28.5	34
Abdominal aorta	14.5	16.5	18.5	13	13	19.5	18	18	18.5	16	20	21.5	23.5	29.5
Age of Patients	2 d.	14 d.	6 w.	2 m.	2½ m.	5 m.	5 m.	7 m.	7½ m.	18 m.	21 m.	3 y.	4 y.	10 y.
Patient	259	251	253	246	254	261	260	240	255	244	248	252	241	245

While at 2 and 14 days of age the circumference of the upper abdominal aorta is less than that of the thoracic aorta, at 6 weeks it is a little greater, being again less at 2½ months. At 7 and 7½ months, the rate between the two circumferences is more or less similar to that noted at 2 and 14 days, and becomes considerably less in the abdominal aorta at 18 months, and this difference is maintained, though not so marked, at 3, 4 and 10 years of age. Sometimes the



TEXT-FIGURE 2

Graphic representation of Table III

circumference of the thoracic and the upper abdominal aorta increases more or less regularly. At other times, that of the abdominal aorta grows more rapidly, almost reaching that of the thoracic; while at still other times the growth of the thoracic portion is considerably more rapid than that of the abdominal. Later it becomes slower again as compared with that of the upper abdominal aorta.

tribution is the result of a local disturbance of the blood supply. As a matter of fact, one should expect a more uniform distribution if these were the result of toxemia alone. In Cases 246, 244, 248, 252, 241 and 238, no fatty changes were found, while quite marked sloping overgrowths existed in some of them.

This brings forth the question of juvenile arteriosclerosis. The aorta of Case 240 was the first examined, and after a comparative study of sections from various parts of the aorta a diagnosis of arteriosclerosis was made on the presence of a subendothelial thickening of the intima with some fatty change. This diagnosis seemed logical until I examined seventeen other children dying from diverse infectious and nutritional diseases, finding very similar changes. Transient pachymenia of the intima, therefore, merits consideration as a pitfall in the diagnosis of juvenile arteriosclerosis in infants.

Another pathological finding was a peculiar degeneration of the chromotropic connective tissue. In some places it appears increased in amount and presents a flocculent aspect. The metachromatic color reaction suggests a close relationship with mucoid material, as well as of the chromotropic substance itself. However, in some investigations preceding those of Björling¹³ and of Schultz,¹⁴ the chromotropic connective tissue was considered and described as mucoid degeneration, inasmuch as it was then generally assumed that the normal connective tissue of the vessel did not give the reaction for mucin.

In order to avoid a misunderstanding in regard to the peculiar change above referred to, the term flocculation has been employed. Flocculation (mucoid degeneration, hyaline degeneration) of the chromotropic substance is better evidenced in frozen sections stained by cresyl violet. It is completely overlooked in ordinary sections of material embedded in paraffin and stained by hematoxylin-eosin. Therefore, while metachromasia of the vascular connective tissue may be made apparent in paraffin sections, as Schultz has pointed out, such is not the case with the delicate pathological change above described.

The study of frozen sections by the Schultz stain suggests that in childhood the elastic substance is not deposited at the same time in all portions of the elastic tissue of the aorta. At first it is manifest in small, irregular segments (Figs. 1 and 7) which have lost their meta-

disturbances and infectious disease. In Case 253, for example, a girl 6 weeks old, who developed a cerebral abscess following an infected spina bifida, and in whom the infectious process was present during practically all of her extra-uterine life, marked and extensive thickenings of the intima were found. They were less marked in Case 264, a well developed and well nourished 7 months old baby dying of leptomeningitis after eight days of illness. But, on the other hand, they were also quite distinct in another well developed and well nourished baby (Case 252), 3 years old, dying of diphtheria on the fifth day of illness.

Why do the sloping overgrowths develop just at the posterior border of the descending aorta? Robertson¹² demonstrated that the vascularization is different in the ascending aortic limb and in the descending thoracic portion of the aorta. While an anastomosing circle of vessels exists in the ascending aortic limb, so that the vascularization is assured in a more or less uniform manner at the front, back and lateral portions of the aorta, this is not the case with the descending thoracic aorta. In the latter the vasa vasorum are most numerous along two longitudinal strips just lateral and parallel to the efferent vessels, so that the vascularization is distinctly more developed at the back than at the front and lateral parts. It is obvious that the subendothelial layer of the intima will show a tendency to increase more quickly in the better-nourished portions of that coat, a direct relation existing between growth of connective tissue and the blood supply. Apparently, as a developmental change, this becomes particularly prominent during infancy.

In thirteen of the twenty cases, I found fatty changes in the intima. In a few instances they presented no relation to developmental thickenings (Cases 264, 255, 262 and 245). Fat occurs more or less uniformly throughout the lining endothelial cells, or as extra-cellular fine droplets along the elastica interna. More often, however, fat occurred in the form of anisotropic droplets (lipoid degeneration) in star-shaped and fusiform cells more or less abundant in the sub-endothelial layer.

In Cases 263, 254, 1, 261 and 240, fatty and lipoid degenerations were more marked and sometimes sharply limited to the sloping overgrowths. The degenerative processes appeared focally in the aorta in the same localities in which fatty streaks and early arteriosclerotic changes later became predominant. Possibly such focal dis-

tribution is the result of a local disturbance of the blood supply. As a matter of fact, one should expect a more uniform distribution if these were the result of toxemia alone. In Cases 246, 244, 248, 252, 241 and 238, no fatty changes were found, while quite marked sloping overgrowths existed in some of them.

This brings forth the question of juvenile arteriosclerosis. The aorta of Case 240 was the first examined, and after a comparative study of sections from various parts of the aorta a diagnosis of arteriosclerosis was made on the presence of a subendothelial thickening of the intima with some fatty change. This diagnosis seemed logical until I examined seventeen other children dying from diverse infectious and nutritional diseases, finding very similar changes. Transient pachymenia of the intima, therefore, merits consideration as a pitfall in the diagnosis of juvenile arteriosclerosis in infants.

Another pathological finding was a peculiar degeneration of the chromotropic connective tissue. In some places it appears increased in amount and presents a flocculent aspect. The metachromatic color reaction suggests a close relationship with mucoid material, as well as of the chromotropic substance itself. However, in some investigations preceding those of Björling¹³ and of Schultz,¹⁴ the chromotropic connective tissue was considered and described as mucoid degeneration, inasmuch as it was then generally assumed that the normal connective tissue of the vessel did not give the reaction for mucin.

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chromatic color reaction. As other segments in the same membrane retain their original metachromasia, a discontinuous appearance is obtained. Elastic membranes in which the elastic substance is incompletely formed were more abundant in the internal and external than in the middle portions of the media (Fig. 7) and more abundant in the ascending and transverse than in the thoracic and abdominal aorta. The elastica interna presents a discontinuous aspect (Fig. 1) in all the children examined, and its internal surface is more or less irregular. Such appearances are related to the age: the younger the subject, the more marked the granulation (compare Figs. 1 and 7).

SUMMARY AND CONCLUSIONS

In childhood the intima of the aorta presents a variable thickness in the same subject, if complete transverse sections from the ascending, transverse, thoracic and abdominal aorta are examined.

At the posterior part of the aorta, the intima often measures more than 100 microns in thickness, resulting from the presence of a cellular subendothelial layer. The thickened portions of the intima, however, do not rise above the surface of the vessel, so that they cannot be detected macroscopically. The intima in transverse sections becomes gradually thickened, until a maximum is attained in its posterior portion. Beyond this, it again becomes gradually attenuated and terminates in tapering ends as the subendothelial layer disappears. The peculiar aspect is referred to as "sloping overgrowths" of the intima.

A comparative study of such changes in a group of twenty children between the ages of 2 days and 10 years suggests that they correspond to transient and recurrent developmental changes. They were regularly found in eighteen children, lacking only in two infants, 2 and 14 days old.

These postnatal developmental changes do not present any definite relation to the age of the subject, to the location in the aorta or to degenerative and non-specific inflammatory processes.

It is assumed that they are not permanent but transient physiological changes, so that quite marked thickenings (*pachymenia*) of the intima tend to disappear soon after they are produced and as the growth of the aorta proceeds. At the same time others arise in other portions of the vessel. It seems likely that these reactions are reproduced many times in the same subject.

Differences in the rate of growth between the subendothelial layer and the vessel as a whole, and between two adjoining portions of the aorta, appear to be the chief factors in the production of the transient pachymenia of the intima in infancy.

These results also confirm the frequency, in children, of fatty changes in some localities of the aorta, namely, the mouth of the intercostal arteries and the ascending and the abdominal aorta, previously reported by Oppenheimer,¹⁵ Klotz and Manning,¹⁰ Stumpf,⁹ Klotz,^{16, 17} Schmidtman,¹⁸ Zinserling,¹⁹ Kube and Ssolowjew,²⁰ and others. They show, moreover, that flocculation (mucoid degeneration) of the chromotropic connective tissue, involving especially the internal portions of the media in the ascending and transverse aorta, is another finding in infants dying from infectious diseases, and that fatty and lipoid degeneration may occur focally in connection with a dorsal connective tissue or developmental plaque.

Developmental changes associated with degenerative processes are a pitfall in the diagnosis of juvenile arteriosclerosis in infancy. In some children fatty changes were seen in the absence of developmental thickenings of the intima, and in others, on the contrary, quite marked simple developmental changes were found in the absence of degenerative processes.

In conclusion, the writer wishes to express his thanks to Professor Oskar Klotz for much helpful advice and guidance during the progress of this work in the Department of Pathology of the University of Toronto.

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DESCRIPTION OF PLATE

All figures were drawn with camera lucida. Only the homogeneous greenish segments containing elastic substance are represented in each elastic membrane in Figs. 1 and 7. The outlines of the coats of the aorta and the elastica interna are the only structures represented in Figs. 2, 3, 4, 5, 6, 8 and 9. I = intima; M = tunica media; Ei = elastica interna.

PLATE 83

- FIG. 1. Case 259, infant 2 days old. Elastic tissue (Schultz' stain) in thoracic aorta; a = elastica interna; b = elastic membrane from the middle of the media; c = elastic membrane from the external portion of the media.
- FIG. 2. Case 263, infant 7 weeks old. Complete transverse section of the abdominal aorta 3 mm. above the origin of the common iliacs. The subendothelial layer becomes gradually thicker, reaching a maximum at the back part of the vessel. This dorsal thickening of the intima is referred to as "sloping overgrowth."
- FIG. 3. Case 254, infant $2\frac{1}{2}$ months old. Sloping overgrowth at the back part of the thoracic aorta between the fourth and fifth pair of intercostal arteries.
- FIG. 4. Case 254, infant $2\frac{1}{2}$ months old. Marked sloping overgrowth in the back part of the abdominal aorta at the mouth of the left renal artery. In this complete transverse section, the subendothelial layer appears discontinuous and the thickenings usually present near the mouth of a branch form a structure quite apart from the dorsal connective tissue plaque.
- FIG. 5. Case 254, infant $2\frac{1}{2}$ months old. In this complete transverse section of the abdominal aorta 5 mm. above the common iliacs, the subendothelial layer is continuous, forming a marked sloping overgrowth at the back part.
- FIG. 6. Case 261, infant 5 months old. The edge and initial portion of three sloping overgrowths are represented, one, the most marked, at the thoracic aorta (a); another, less marked, at the upper abdominal aorta (b); and still another at the lower abdominal aorta (c).
- FIG. 7. Case 261, infant 5 months old. Elastic tissue in thoracic aorta (Schultz' stain); a = elastica interna; b = elastic membrane from the internal portion; c and d = middle portion; and e = external portion of the media.
- FIG. 8. Case 248, infant 21 months old. Sloping overgrowth at the back part of the upper abdominal aorta (mouth of the celiac axis).
- FIG. 9. Case 262, child 5 years old. At the thoracic aorta, the intima presents a subendothelial layer of more or less uniform thickness.

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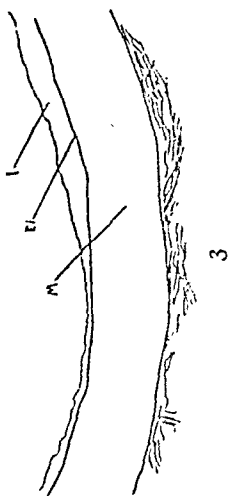
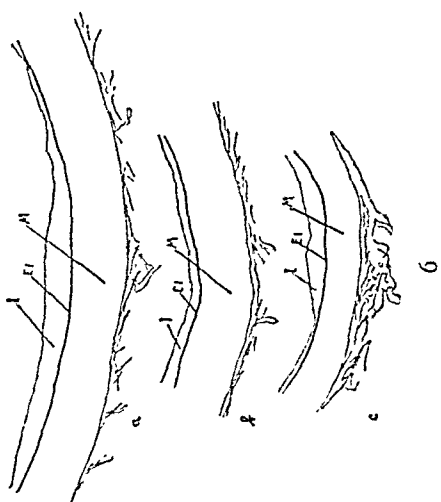
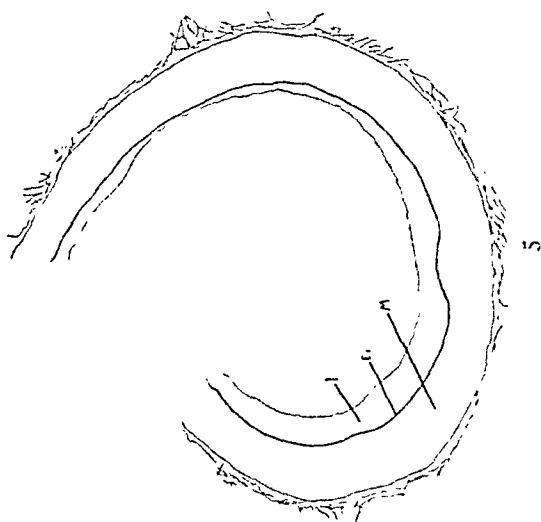
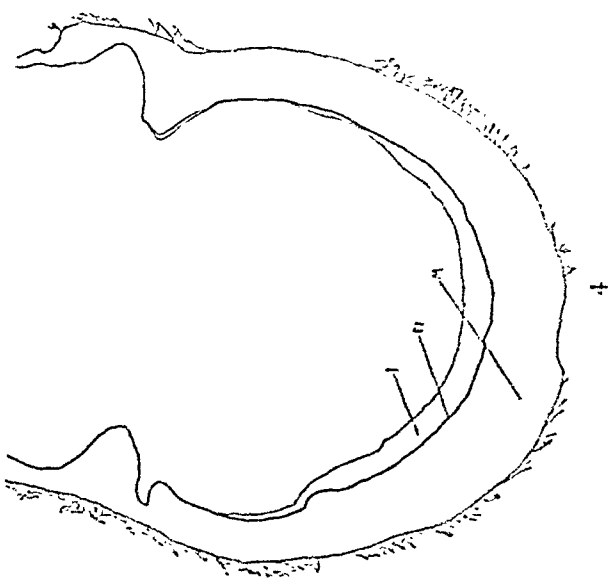
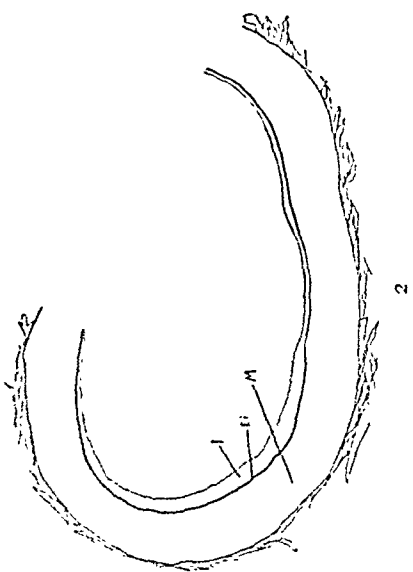


Figure 2 shows the dopa reaction of normal Caucasian skin. In the colorless derma are a few black leucocytes. In most sections of normal skin there are a few scattered leucocytes; they have no significance. The chief point of interest is the blackening of certain cells in the basal layer of the epidermis and in the matrix of the hair — that is to say — the sites of melanin production. These are the melanoblasts revealed by the dopa reaction.

The melanoblasts of the epidermis assume many shapes — round, cuboidal, columnar and branched. Traditionally the branched cells of the epidermis are called dendritic. Often they contain melanin, often not. Their common property is the ability to oxidize dopa to melanin, blackening in dopa solutions. Bloch holds that in this dopa-oxidase which converts dopa to melanin he has discovered the agent which manufactures natural melanin in mammalian skin. The dopa reaction thus becomes an indicator of the presence of the natural oxidase. Leucocytes being excepted, it follows that every dopa-positive cell is an active melanoblast.

After many experiments on all sorts of tissue, normal and pathological, pigmented and non-pigmented, I accept Bloch's view as the most satisfactory working hypothesis yet offered to explain the production of melanin in human skin. The illustrations will show some of the evidence which led to this conclusion.

Our material consisted of freshly excised surgical specimens from many parts of the human body. Our first and fundamental conclusion was that, leucocytes being excepted, dopa-positive cells are found only in those tissues where melanin is being produced, or where it can be produced under appropriate stimulation. These tissues normally are the skin and the mucous membranes of ectodermal origin; pathologically they are pigmented moles and malignant melanomas.*

MELANIN IN EXCESS

Having agreed with Bloch that dopa-positive cells are found only in melanin-producing tissue, in our next finding we were obliged to

* The pigment of the eye constitutes a special field which has been little cultivated. Since the dopa reaction requires fresh tissue, or tissue that has been fixed in 5 per cent formalin for five hours at the longest; and since it is customary to fix specimens of human eye much longer than that, there are practical difficulties in attempting dopa reactions with this material. A thorough study of the dopa reaction of the embryonic eye was made by Miescher.²³

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MELANOMA STUDIES *

I. THE DOPA REACTION IN GENERAL PATHOLOGY

GEORGE F. LAIDLAW, M.D.

(From the Laboratories of the Department of Surgery, College of Physicians and Surgeons, Columbia University, New York, N.Y.)

Introduced in 1917 by the distinguished dermatologist of Zurich, Bruno Bloch,¹⁻⁴ and defended by him vigorously ever since,⁵⁻¹⁵ the dopa reaction has been used chiefly by the dermatopathologist (Becker,^{16, 17} Peck,¹⁸⁻²⁰ and Kissmeyer^{21, 22}). It deserves to be more widely known and practiced both by the histologist and by the general pathologist. Following the simple method devised by Blackberg, which is described in detail in the second paper of this series, the dopa reaction ** can be carried out easily and accurately in any laboratory.

The dopa reaction is specific for two kinds of cells, for melanoblasts (a term which includes all melanin-producing cells as distinguished from mere phagocytes), and for myelogenous leucocytes (cells which have no known connection with melanin production). Both of these cells contain a ferment, an oxidase, which converts dopa to melanin. The newly formed melanin colors the cell black. This blackening of the reacting cell is the dopa reaction. The ferment of the melanoblast is dopa-oxidase — specific for dopa — it oxidizes nothing else. The ferment of the leucocyte is a polyphenol-oxidase, oxidizing many phenols to colored products.

Figure 1 shows the dopa reaction of a Hodgkin's lymph node. The black spots are the eosinophil and neutrophil leucocytes. Lymphocytes and collagen are colorless.

* Received for publication April 22, 1932.

** The word "dopa" is Bloch's abbreviation for 3, 4-dioxyphenylalanin.

and in the moles illustrated, and in malignant melanomas, the active melanoblasts may be of any shape — round, cuboidal, stellate or dendritic. The shape of the cell has absolutely nothing to do with its melanin-producing power or with its malignancy. Figure 9 shows the dopa reaction of another pigmented mole removed from the arm of a negress. This unusual mole consists exclusively of dendritic cells, all of them dopa-positive. The clinical history of such moles differs in no way from that of moles composed of round or other non-dendritic cells.

MELANIN ABSENT

Having become convinced that an increase in the number of dopa-positive cells goes hand in hand with increased activity of pigment formation, we studied the opposite condition — decrease and absence of melanin. Figure 10 shows the dopa reaction from the edge of a patch of vitiligo of negro skin. On the left is seen normal pigmented negro epidermis with its dopa-positive cells. Then comes a zone of bizarre dendritic cells, as is usual at the margin of pigmentation or depigmentation. On the right the colorless skin begins. Here the dopa-positive cells have disappeared, together with the melanin. This disappearance of the dopa-positive cells in vitiligo was described first by Bloch,²⁴ who reported a similar disappearance of dopa-positive cells from the hair matrix of graying hair.²⁵

Figure 11 shows the dopa reaction from the edge of a black spot on the white ear of a guinea-pig. Melanin and dopa-positive cells are abundant in the black spot, totally absent from the white skin. Bloch⁷ and his school have shown repeatedly that there are no dopa-positive cells in albino skin and that no amount of radiation will elicit either melanin or dopa-positive cells in such skin, although in non-albino skin it is a simple matter to produce both of them by radiation (Lutz²⁶). Thus, whether the absence of melanin be congenital (albino) or acquired (vitiligo), where there is no melanin there are no dopa-positive cells; or, to put it the other way, where there are no dopa-positive cells no melanin is made.

MELANIN REAPPEARS

The final proof that dopa-positive cells are concerned in melanin production is that they are the invariable precursors of melanin's appearance or reappearance. Among dermatologists it is well known

agree with him again that wherever melanin is being produced in excess, there the dopa-positive cells are increased in number and often in complexity of dendrites also. Figure 3 is the dopa reaction of normal negro skin from the breast. The dopa-positive cells are more numerous than in the normal Caucasian skin of Figure 2. In this negro skin most of the dopa-positive cells are round and devoid of dendrites, negating the common belief that a melanoblast must be dendritic. Figure 4 shows circumanal skin from another negro. The dopa-positive cells are numerous; many of them are dendritic. Figure 5 shows the increased number of dopa-positive cells in the epidermis of a dark brown patch of von Recklinghausen's disease in Caucasian skin. All of these melanoblasts are round, without dendrites. Figure 6 shows Caucasian skin that has been tanned by exposure to X-ray. Here melanin production is very active. The dopa-positive cells of the epidermis are numerous. The many leucocytes in the corium are an expression of the X-ray dermatitis.

Thus, it matters not whether the pigmentation be racial (negro), or pathologically congenital (von Recklinghausen) or acquired, or a reaction to radiation from without (sunlight, radium, thorium, X-ray), the increased production of melanin is attended by an increase in the dopa-positive cells.

Figure 7 shows the dopa reaction of a pigmented mole, illustrating Bloch's conception of the distinction to be made between active and latent melanoblasts. This distinction will be discussed under the caption Latent Melanoblasts. In the upper zone of this mole there is abundant melanin; here the nevus cells are strongly dopa-positive. Passing downward there is less and less melanin and correspondingly the dopa reaction grows fainter and soon disappears. Three-fourths of the cells in this nevus are producing no visible melanin; these cells are dopa-negative. The limitation of melanin production to the upper layers of a pigmented mole is common. It is seen again in Figure 8. In both of these moles the melanin-producing cells are round and devoid of dendrites.

DENDRITIC CELLS

The common belief that melanoblasts must be dendritic is an error carried over from the frog histology which has long dominated human melanogenesis. It is true that active melanoblasts are often dendritic, but they are not necessarily so. In the pigmented skins

man "buccal mucosa" (site not stated). Becker¹⁶ found both melanin and dopa-positive cells in Adachi's locations and added the normal mucous membrane from the middle of the cheek and from the pharynx at the level of the hyoid bone. Laidlaw and Cahn³⁵ found dopa-positive cells and melanin in normal human gum (Fig. 12); Laidlaw (unpublished) in normal anal canal. The presence of melanoblasts explains the occurrence of primary melanoma in these mucous membranes and also the absence of primary melanoma in non-ectodermal mucous membranes which normally harbor no melanoblasts and make no melanin.

In blonds, melanin and dopa-positive cells of the mucous membranes are scanty, as they are in blond skin; in the mucous membranes of negroes and brunets, they are abundant, an observation already made by Adachi in regard to melanin. To the naked eye, such a mucous membrane may look pink and without a trace of pigmentation; nevertheless, on microscopic examination melanin will be found in abundance. Ramel noted racial pigmentation of the mouth in Tziganes; Cahn observed similar racial pigmentation of the mouth in negroes and in a negroid type of Bavarian Jew.

CHROMATOPHORES OF THE DERMA

In the upper layers of the derma of every normally pigmented skin there are seen cells of various shapes containing melanin. These cells never give the dopa reaction. Consequently according to the dopa hypothesis they contain no oxidase and they cannot have produced the melanin which they contain. Often these cells are obviously phagocytic; they seize and retain any pigment that happens to enter the skin, such as tattoo pigment, blood pigment and gunpowder. Miescher³⁶ proved their phagocytic power for melanin and their long retention of it by injecting melanin into living human skin and excising bits of skin at various intervals afterward. To distinguish them from melanoblasts, these cells are called pigment carriers, chromatophores.

Within certain limitations, to be mentioned presently, the dopa reaction is the one reliable method of distinguishing the melanoblast from the chromatophore. This distinction may be seen in any negro skin or pigmented Caucasian skin. Miescher³⁷ and von Albertini and Walthard³⁸ have used the dopa reaction to identify melanoblasts in the metastases of malignant melanoma.

that some patches of vitiligo can be stimulated temporarily to repigmentation by exposure to radiation (Buschke,²⁷ Buschke and Mulzer,²⁸ With²⁹). In such repigmentation of vitiligo, both Bloch and Kissmeyer³⁰ have reported that the dopa-positive cells reappear first; melanin follows. In my own observations of the repigmentation of scars, this sequence is invariable; it is especially striking in negro skin. Bloch^{7, 25} has described the same sequence in the human embryo, Miescher²³ in the eye of the embryo chick, rabbit and guinea-pig, Peck¹⁹ in tanning of human skin by thorium radiation. The dopa-positive cells appear first, melanin next, never in the reverse order. The inference is that the dopa-positive cells are essential to the production of melanin.

THE ACANTHOSES

In his early experiments Bloch soon found dopa-positive dendritic cells to be abundant among the epithelium of the acanthoses. The word is Unna's; it applies to the thickening of the prickle-cell layer of the epidermis seen in psoriasis, papilloma, condyloma and similar lesions. Usually there is no excessive pigmentation, often no melanin whatever. This observation has been confirmed by all dopa workers. I have seen it repeatedly. In fact, the massing of dopa-positive dendritic cells a little distance back from the margin of a granulating wound might be ascribed to the acanthosis always present in this zone quite as justly as to their being forerunners of pigmentation.

According to Kyrle,³¹ that rare spirit too early lost to dermatopathology, the cells of the epidermal basal layer have two functions, melanin formation and proliferation. It seems that stimulation of either function increases the number and complexity of the dopa-positive cells. I have been unable to correlate this acanthotic increase of these cells with their pigment function. This phase of their activity awaits adequate explanation.

MUCOUS MEMBRANES

Among mucous membranes, production of melanin is confined to those of ectodermal origin. Adachi³² found melanin in the epithelium of the mucous membrane of the cheek, lower lip, prepuce and vagina. He was limited to hand-cut, unstained sections. Using both the silver and the dopa reaction, Redslob³³ found melanin and dopa-positive cells in normal human conjunctiva, Ramel³⁴ in normal hu-

MONGOL CELLS

These ribbon-like cells buried deep in the corium over the sacrum and along the backs of infants of all races are true melanoblasts, the only melanoblasts of mesodermal origin in normal human skin. Bloch⁷ and Bahrawy⁴¹ found them to be dopa-positive. As an anomaly I saw them in the neurofibromatous skin of von Recklinghausen's disease from over the sacrum of a Caucasian girl 18 years of age. As shown in Figure 13, they were dopa-positive. They blackened with silver also, proving their scanty pigment to be melanin. Melanomas arising from these cells and from their analogues, the cells of blue nevi, are the only melanotic tumors of human skin to which the name melanosarcoma can justly be applied, a view first formulated by Darier,⁴² prompted by a suggestion from Bloch.

Not every nevus that looks bluish black conforms histologically to the Tièche-Jadassohn blue nevus.⁴³ Even epidermal melanin in the tips of long rete pegs will look bluish and not brown if there is little or no melanin in the surface epithelium (Fig. 12). Sato⁴⁴ has made a similar observation of ordinary nevus nests that were deeply placed in the corium. The diagnosis of blue nevus, and the sequent melanosarcoma, should rest on microscopic examination.

SARCOMA AND CARCINOMA

The dopa reaction has no relation to malignancy, as such. In repeated tests of various forms of non-melanotic sarcoma and carcinoma, I have found the tumor cells to be consistently dopa-negative.

EPITHELIOMA

Recalling Kyrle's dictum that the function of the epidermal basal cells is twofold, proliferation and melanin production, it might be expected that the progeny of these cells in forming an epithelioma would show some melanoblastic characteristics, especially since acanthosis or thickening of the prickle-cell layer is attended by an increase in the dopa-positive cells. This expectation is fulfilled. As in all tumors, function is performed imperfectly and irregularly. In some epitheliomas I find no dopa-positive cells; the dopa-positive cells of the overlying skin stop some distance back from the edge of the ulcer as they do in non-malignant granulating wounds. In other

LATENT (DOPA-NEGATIVE) MELANOBLASTS

The use of the dopa reaction as a specific stain for melanoblasts stumbles over the difficulty that the cell capable of producing melanin is not always and everywhere dopa-positive; it does not always contain the oxidase. This is seen readily in pigmented moles and in melanomas where broad areas of the tumor are free from visible melanin and from dopa-positive cells. Here a negative reaction means nothing; only the positive cells count. (The situation is different with the chromatophores of ordinary pigmented skin. Thousands of microscopic sections examined by competent dopa workers have never once revealed a positive dopa reaction in the chromatophores of the upper derma. We may accept them as permanently lacking the melanin-producing oxidase).

In his first publication, Bloch¹ wrote that in malignant melanoma the cells around the growing margin of the tumor were most apt to be dopa-positive, while many of the cells in the center of the tumor were negative — an observation confirmed by Miescher³⁹ and by von Albertini and Walthard.³⁸ Both Bloch and Miescher point out that the dopa-positive melanoblast, whether dendritic or non-dendritic, does not always contain melanin, neither is the melanin-containing cell always dopa-positive. These discrepancies are explained best by Bloch's ferment hypothesis, according to which a distinction must be made between the oxidase (which carries out the dopa reaction), and the finished melanin (which does not carry out the reaction). The dopa-positive factor — the oxidase — appears in the cell some time before the resulting melanin is visible (see caption Melanin Reappears); the melanin itself may remain in the cell long after the oxidase has disappeared (Miescher's experimental injection of melanin into human skin).

My own experience is in strict harmony with the ferment hypothesis; but, whatever the explanation, there remains the fact that the dopa reaction must be used with this precaution, that not all cells capable of producing melanin are at all times dopa-positive. In her studies of pigmentation of the skin of the embryo and newly born gray mouse, Steiner-Wourlich⁴⁰ found that even in this normal, progressive pigmentation the production of melanin is not continuous, and the dopa reaction is not continuously present; there are intervals of rest.

mel⁴⁷ and Meirowsky.⁴⁸ My own experience, covering hundreds of sections of skin stained with various silver techniques, and additional hundreds of dopa sections compared with silver staining of the same skin, agrees absolutely with Bloch's statement that the dopa reaction and the silver reaction are totally different things. To an experienced dopa worker it is obvious that Bloch's critics have been misled by overstained dopa sections. The correct reactions could never be mistaken for one another. Silver blackens melanin wherever found, in the derma as in the epidermis, in melanoblasts, phagocytes, and free in the lymph spaces indifferently. If the dopa-positive cells happen to contain melanin they will stain with silver; otherwise not. Dopa, on the other hand, singles out the active melanoblasts, leaving the melanin in the resting cells unstained. The only melanin that blackens with dopa is the melanin inside of a dopa-positive cell, on which the dopa-melanin is adsorbed or deposited.

THE DIMETHYLPARAPHENYLENDIAMIN CONTROVERSY

On the publication of the dopa reaction Kreibich⁴⁹ declared that he had long secured similar effects from dimethylparaphenyldiamin (one of the components of the Schultze-Winkler formula), a position in which he is supported by Meirowsky,⁴⁸ who goes even further and finds the dopa reaction duplicated by a whole series of easily oxidizable phenols. After many trials with this most highly praised phenol of this group, I agree with Bloch¹² and with Walthard⁴⁵ that this phenol, like silver, stains only the melanin and not the protoplasm of the cell. Even when the reaction succeeds, the demonstration of the dendritic cells is far inferior to that of the dopa reaction, as Meirowsky himself admits.

THE MAST CELL CONTROVERSY

Of the many controversies prompted by the dopa reaction, the oddest and the least necessary would seem to be the difference of opinion of equally well qualified observers over the reaction of mast cells. That master of dermatopathology, P. G. Unna, wrote long ago that mast cells abound in the snout of the white rat and in human neurofibroma. It is a simple matter to immerse fresh frozen sections of these tissues in dopa solution and counterstain them with cresyl

epitheliomas the dopa-positive cells continue in the surface epithelium over the tumor, or a few scattered dopa-positive cells are seen among the tumor epithelia, chiefly in the basal layer. As in the acanthoses, these cells are mostly dendritic. Their presence in an epithelioma seems to have no significance.

MELANOSIS COLI

In melanosis coli the tunica propria of the mucosa contains many large, round, stellate and spindle cells loaded with yellow-brown granules. In the specimens which I have examined the pigment reacts like melanin in that the fine granules blacken quickly in silver, while the larger granules require a long time, perhaps several days. Current opinion is divided as to the nature of the pigment, but is agreed that it has been absorbed from the intestinal contents and phagocytized by these cells. The view that the cells are phagocytes is corroborated by Walthard's⁴⁵ finding them dopa-negative at autopsy.

Negative dopa reactions of autopsy material are open to suspicion, owing to the length of time that necessarily elapses between death and the immersion of the tissue in dopa. By the kindness of Doctor Janssen, I was able to immerse a specimen of diffuse melanosis of the upper part of the rectum in dopa within two hours of its excision from the living body. Microscopically the mucosa presented the typical appearance of melanosis coli. The pigment-bearing cells were dopa-negative. There were no dopa-positive cells, except polynuclear leucocytes which abound in this mucosa. I have treated many fresh surgical specimens of colon and rectum with dopa and also tested them for melanin with silver with consistent negative results. The conclusion is that there are no melanoblasts and no melanin in the colon or in the rectum above the mucocutaneous junction. It follows that the occurrence of primary melanoma above this line is highly improbable. If it occurs, it must spring from misplaced islands of ectoderm.

THE SILVER CONTROVERSY

Soon after Bloch's first publications Heudorfer⁴⁶ declared that there is nothing specific in the dopa reaction and that it merely duplicates pigment staining with silver, an assertion endorsed by Lem-

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violet. In such sections it is seen beyond any possible doubt that Bloch scores once again. The mast cell, both in the white rat and in human neurofibroma, is dopa-negative. The reader may see this for himself in Figures 5 and 13, from the same von Recklinghausen neurofibroma. These sections contain myriads of mast cells, not one of which has become visible in the dopa solution.

SUMMARY

1. Bloch's dopa doctrine is endorsed as the best working hypothesis of melanin production in human skin.
2. The dopa reaction is indispensable in the study of pigmented moles, melanoma and the movements of melanin.
3. In the identification of melanoblasts with the dopa reaction, only the positive cells are significant.
4. The appearance of dopa-positive dendritic cells in non-pigmented acanthoses remains unexplained.
5. In the controversies which have arisen over the dopa reaction, Bloch's histological findings are corroborated.

In conclusion I must thank Professor Bloch for his kindness in sending me a portion of his dwindling store of dopa in the beginning of these studies two years ago; Dr. S. N. Blackberg of the Department of Pharmacology, who showed me how simple a matter the dopa reaction could be made to be; Dr. Jerome Webster for many fresh specimens from his plastic surgery clinic; and Professor Purdy Stout who generously places his choicest material at my disposal.

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DESCRIPTION OF PLATES

PLATE 83

All figures are untouched photomicrographs of dopa reactions at pH 7.4 and at 37°C for from 3 to 4 hours.

- FIG. 1. Hodgkin's lymph node. Myelogenous leucocytes black; lymphocytes and collagen colorless.
- FIG. 2. Normal Caucasian skin from breast. In the basal layer of the epidermis there are blackened melanoblasts of various shapes, both round and dendritic. They are few in number and spaced far apart. The rest of the epithelium and the collagen are colorless.
- FIG. 3. Normal negro skin from breast. In the epidermis the dopa-positive cells (melanoblasts) are more numerous than in Fig. 2. Almost all of them are round, not dendritic. The chromatophores of the corium are visible as pale gray spindle cells. They contain no ferment and do not blacken in dopa.

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PLATE 84

- Fig. 2. Normal circumanal skin from another negro. The dopa-positive cells of the epidermis are very black, very numerous and most of them are dendritic. In this corium there are many chromatophores loaded with melanin; some of them blacken in dopa.
- Fig. 3. Dark brown patch of von Recklinghausen pigmentation of Caucasian skin. Melanoblasts of the epidermis very numerous. Almost all of them are round, a few are dendritic.
- Fig. 4. Caucasian skin tanned by X-ray. Dopa-positive cells of the epidermis much more numerous than in the normal epidermis of Fig. 2. In the papillary layer of the corium there are many black leucocytes, an expression of the X-ray dermatitis.

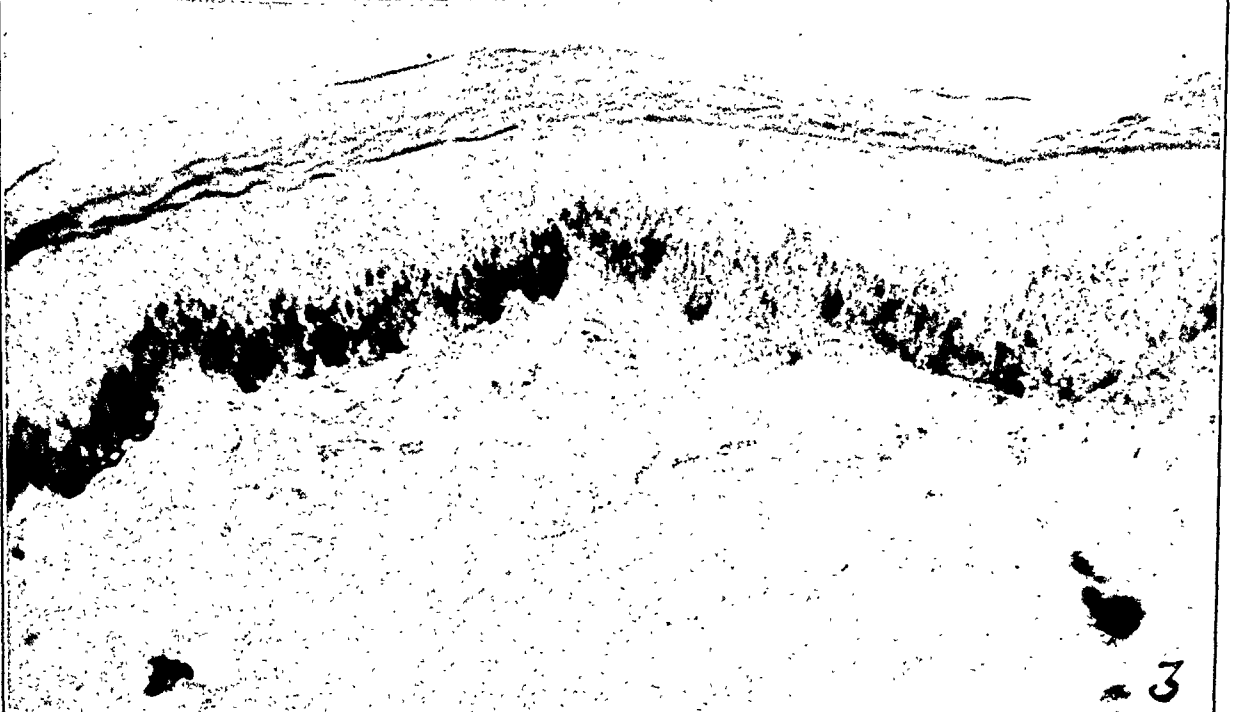
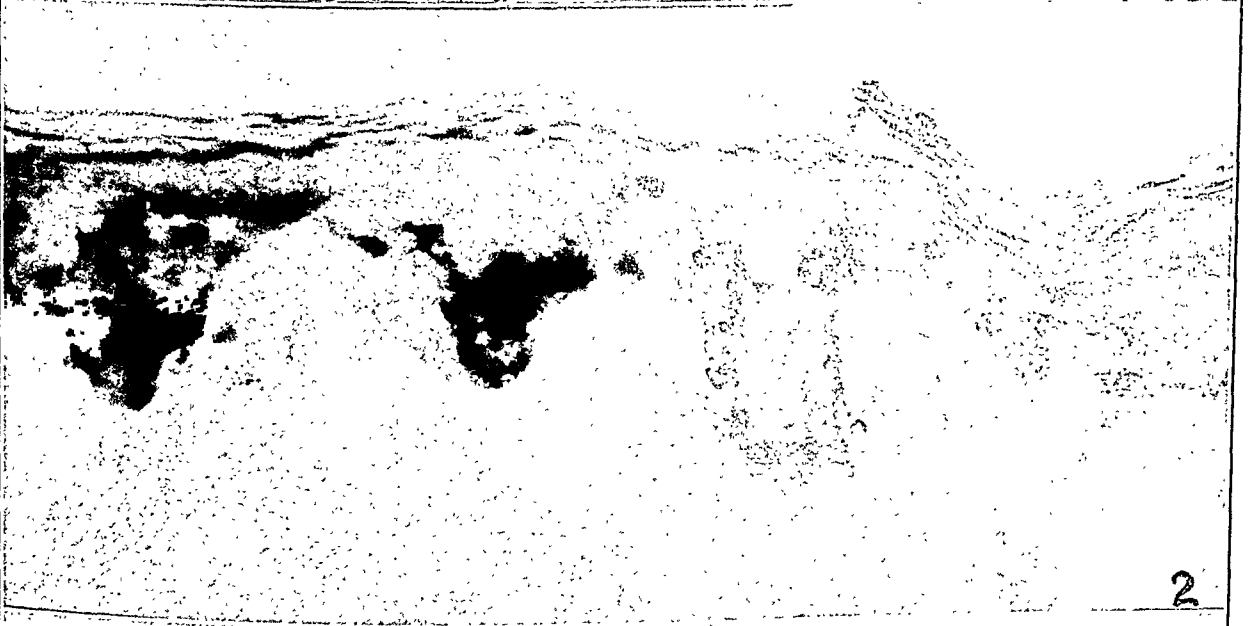
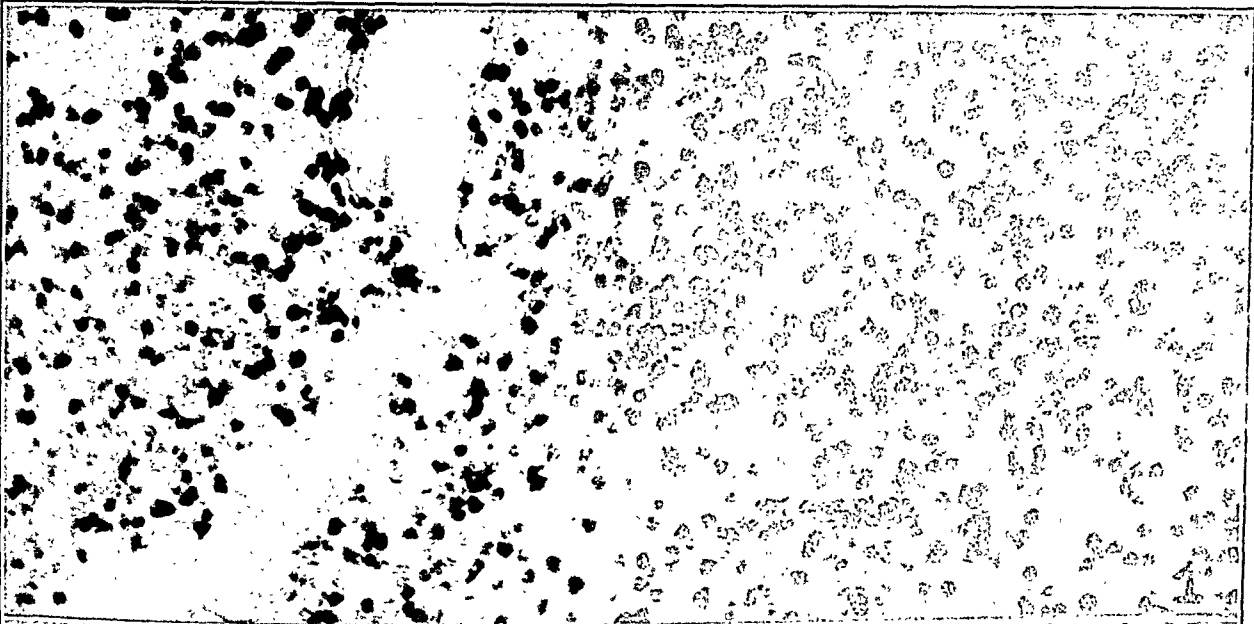


PLATE 85

Fig. 6. Pigmented mole. In the center is a hair in its follicle. There is much melanin in the upper layers of the mole and here the nevus cells are strongly positive, very black. Passing downward the melanin decreases and correspondingly the dopa reaction becomes fainter. In the lower part of the mole where no melanin is being produced, the nevus cells are dopa-negative.

Another pigmented mole with the same features as Fig. 7. In both of these moles the active melanoblasts, the nevus cells, are round and free from dendrites.

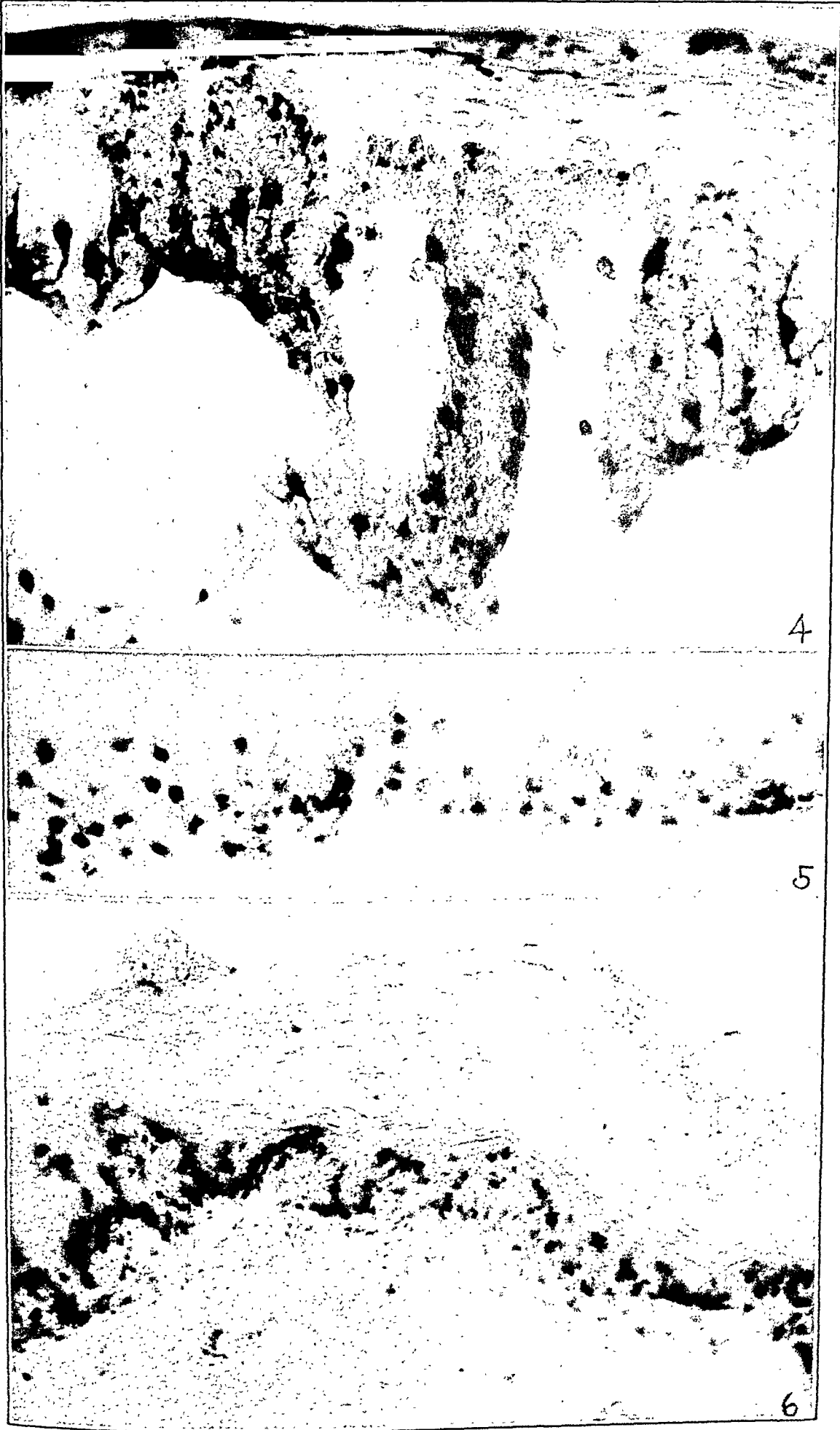


PLATE 86

FIG. 9. Unusual pigmented mole consisting entirely of dendritic cells, all of them dopa-positive. From the arm of a negress.

(Figs. 5, 7, 8 and 9 from patients of Dr. Webster.)

FIG. 10. Margin of patch of vitiligo of negro skin. On the left, normal negro epidermis with abundant melanin and dopa-positive melanoblasts. In the center, the zone of bizarre dendritic cells usually seen at the margin of accumulation or depigmentation of the epidermis. On the right, the depigmented epidermis; both melanin and dopa-positive cells have disappeared. (Patient of Dr. Marie Karelitz.)

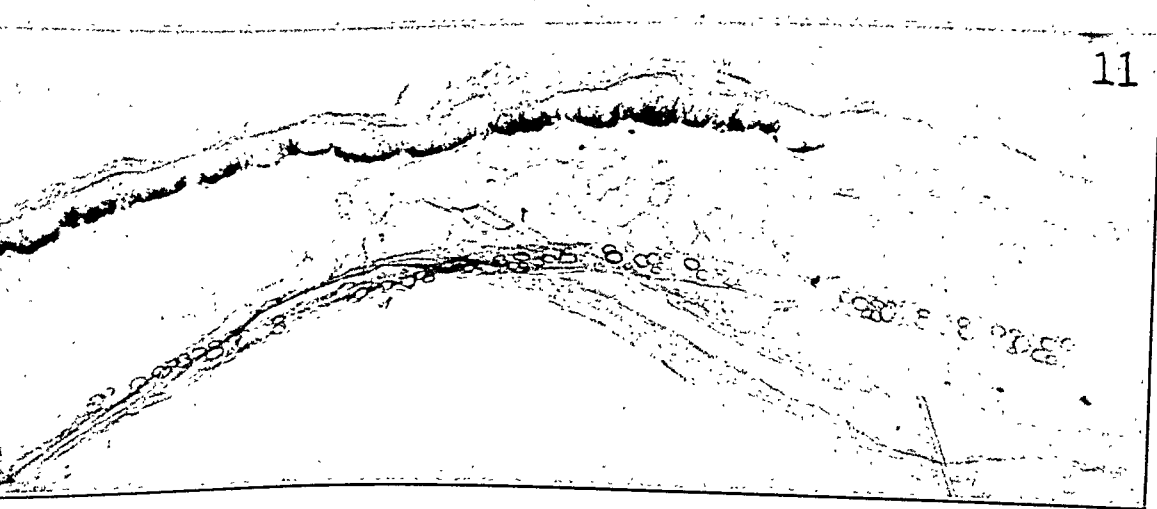
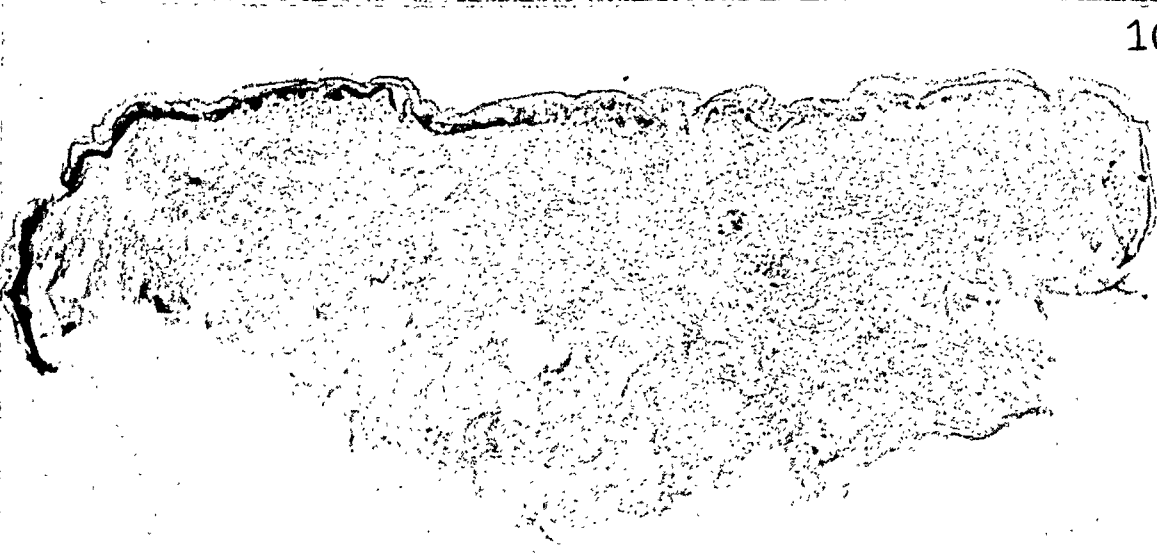
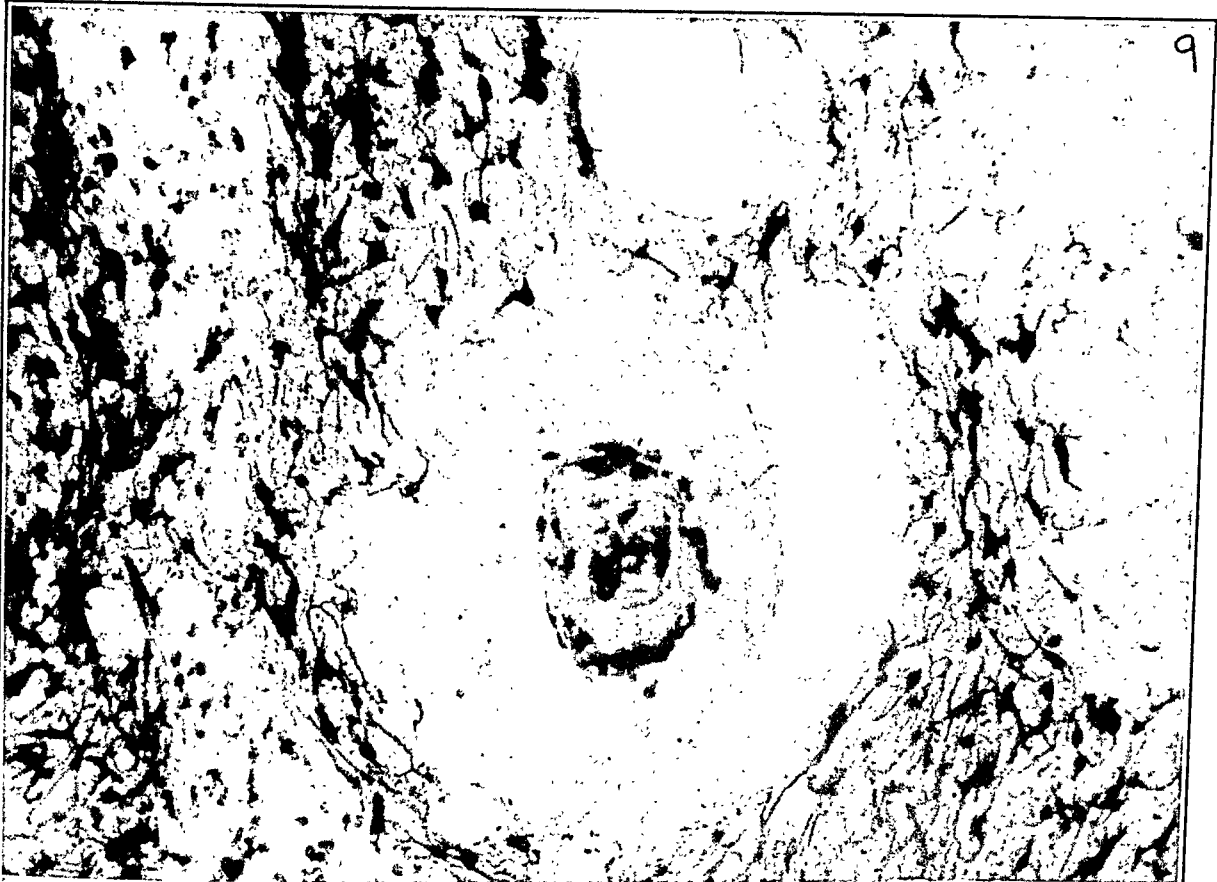
FIG. 11. Edge of black spot on white ear of guinea-pig. In the epidermis of the black spot, melanin and dopa-positive melanoblasts are abundant; they are totally absent from the epidermis of the white skin. At the margin of the black spot, where melanin becomes scanty, the outline of the dendritic melanoblasts is seen more clearly.

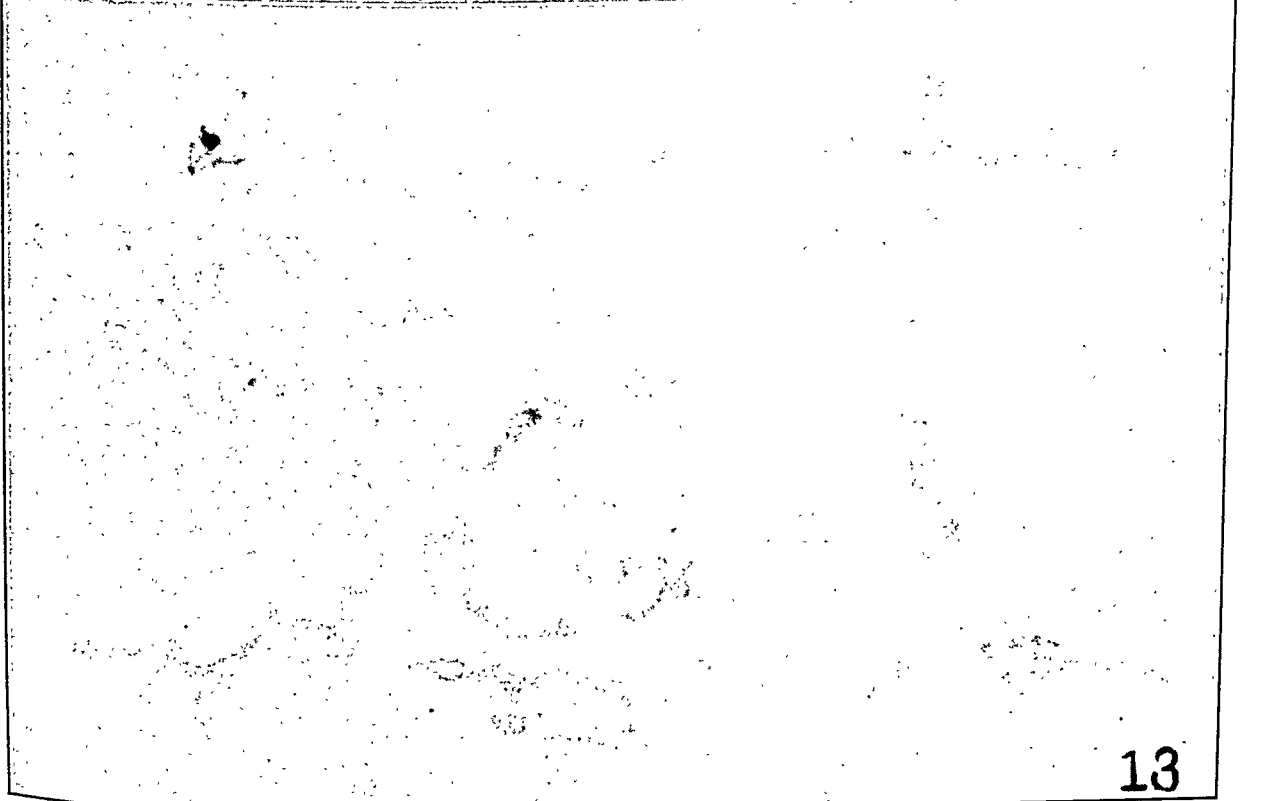
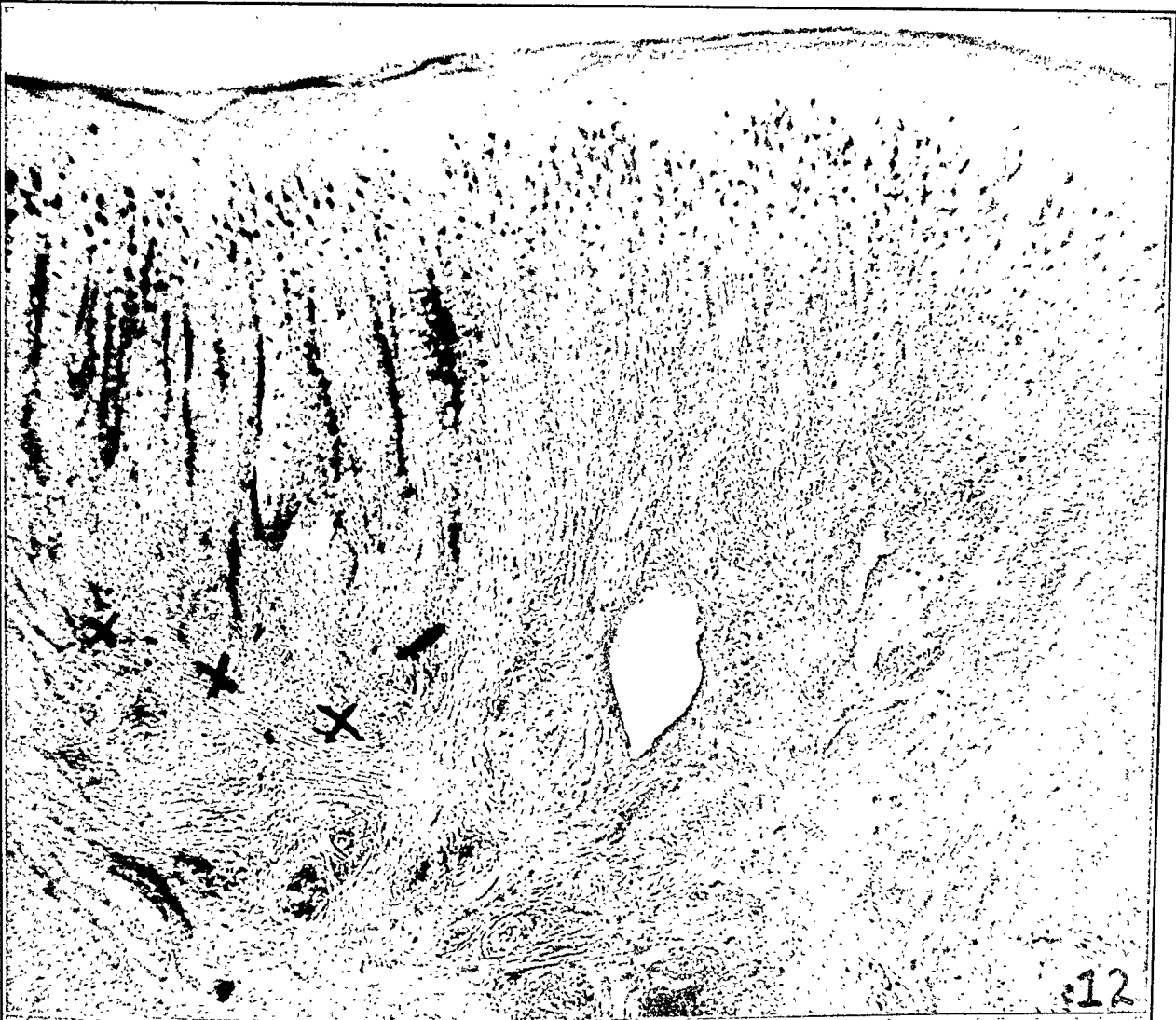


PLATE 87

FIG. 12. Shallow delayed pigmentation of gum of Latin-American; black hair, dark eyes. In the middle third of the very thick stratified epithelium there are many dopa-positive dendritic cells. The melanin itself is situated chiefly in the base of the very long, slender rete pegs. The deep situation of the melanin and the absence of superficial pigment accounts for the blue color of the gum, although the melanin is exclusively in the epithelium and not in the corium, as in the Tièche-Jadassohn blue nevus. An X mark has been placed just below the tips of the longer rete pegs. (Patient of Dr. Lester Cabana.)

FIG. 13. Dopa-positive Mongol cells. Neurofibromatous skin in von Recklinghausen's disease from over the sacrum of Caucasian girl (Polish Jew) 18 years of age. The corium is very thick, averaging 1.5 cm. The groups of ribbon-like cells are situated deep in the corium, in its middle or lower third. They are filled with fine granules of melanin. (Patient of Dr. Webster.)





the dextrorotatory form. In powder, as purchased, dopa keeps indefinitely at room temperature.

Dissolve 0.3 gm. of dopa powder in 300 cc. of cold distilled water. Keep well corked in the refrigerator, where it will remain good for many weeks. The solution is usable as long as it is colorless or only slightly tinged with red. Darker red solutions should be rejected; they oxidize too quickly and overstain the sections.

CORRECTING AN ERROR

Dr. Peck calls our attention to a printer's error that has dogged the steps of the dopa reaction. Bloch uses dopa in a solution of 0.1 of 1 per cent and he has never used anything else. Unfortunately in the literature, even in Bloch's and Peck's own papers, in Romeis' popular Taschenbuch (p. 295), and in Krause's Enzyklopädie der mikroskopischen Technik (Vol. 3, p. 1785), the concentration has been printed incorrectly as from 1 to 2 per cent. Let it be understood then that the correct proportion is 1:1000 and that any statement to the contrary is a printer's error.

THE BUFFERS

Dissolve 11 gm. of disodium hydrogen phosphate ($\text{Na}_2\text{HPO}_4 + 2 \text{H}_2\text{O}$) in 1000 cc. of distilled water.

Dissolve 9 gm. of potassium dihydrogen phosphate (KH_2PO_4) in 1000 cc. of distilled water. Both of these buffers are kept in the refrigerator.

Just before cutting the sections, buffer to 7.4 by adding 2 cc. of the potassium phosphate and 6 cc. of sodium phosphate buffer to 25 cc. of the stock dopa solution. For a small batch of a dozen sections we use 15 cc. of the buffered solution, but we prepare double the quantity immediately required in order to have enough to renew the solution in half an hour. Return the stock dopa solution and the surplus of the buffered solution to the refrigerator immediately; at room temperature the stock solution soon oxidizes and turns red, the buffered solution tends to turn brown.

At a given temperature, the speed of the reaction is determined by the pH. At 7.4 the reaction will be finished in 4 or 5 hours at 37° C. When in a hurry, we hasten the reaction by using only 1 cc. of the potassium buffer, giving a pH of 7.7; or we may omit the potassium

MELANOMA STUDIES *

II. A SIMPLE TECHNIQUE FOR THE DOPA REACTION

GEORGE F. LAIDLAW, M.D., AND SOLON N. BLACKBERG, PH.D.

(From the Laboratories of the Department of Surgery and of the Department of Pharmacology, College of Physicians and Surgeons, Columbia University, New York, N. Y.)

Bloch's dopa reaction is a specific stain for melanoblasts and for myelogenous leucocytes. These cells are believed to contain an organized ferment which oxidizes dioxyphenylalanin (dopa) to melanin. The dopa-melanin colors the reacting cell black. This blackening of the cell is the dopa reaction.¹

In the published descriptions success with the dopa reaction is attributed to a somewhat meticulous observance of certain chemical details. Blackberg proved that many of these precautions can be dispensed with. In fact, we have come to treat the dopa reaction with so little ceremony that when a specimen comes into the laboratory late in the day we put frozen sections in a small vial of buffered dopa in an inside pocket until the solution turns sepia brown, wash the sections in tap water and mount them the next day. However, we prefer to work with a uniform temperature and to control the reaction with the microscope. The following simple method gives constant and accurate results. The reagents required are a stock solution of dopa and the Sorensen buffers.

THE STOCK DOPA SOLUTION

This is a 1:1000 solution of 3, 4-dioxyphenylalanin (abbreviated to dopa) † in distilled water. Dopa is a phenol extracted from *Vicia faba*, a common vetch or sow bean. The levorotatory preparation should be used, since Bloch and Schaaf² and Peck and coworkers³ have shown that melanoblasts have little or no oxidizing power over

* Received for publication April 22, 1932.

† When ordering, specify "for Bloch's dopa reaction." Supplied by the American branch of Hoffmann-LaRoche, Nutley, New Jersey, at 95 cents per gram. With the minute quantity used, the cost of staining a dozen or more sections is less than 2 cents.

Refrigeration: We have tried with little success to preserve melanoblasts in the refrigerator. As with formalin, strongly positive melanoblasts survive for several days; faintly reacting cells disappear overnight.

Leucocytes: The ferment of the myelogenous leucocyte endures much longer than that of the melanoblast. In fact, fresh leucocytes react all the better for a few days in strong formalin and after 2 or 3 months they may still react well. Even for leucocytes there is a time limit, 3 to 4 months, beyond which most of them no longer blacken in dopa solutions.

CUTTING THE SECTIONS

Frozen sections are obligatory since the chemicals of celloidin and paraffin embedding would destroy the ferment. It is important to remember that water extracts the ferment quickly. Neither the block nor the sections should lie in water longer than the few seconds of a quick rinse. Before cutting the sections, the dopa solution should be buffered and poured into dishes ready to receive the sections without delay.

In order to exhibit the long dendrites of melanoblasts some of the sections should be very thick, 75 to 100 microns; others may be from 20 to 30 microns for better detail. Sections of fresh tissue are dropped from the knife directly into dopa. If the tissue has been in formalin, the sections are rinsed for a few seconds in distilled water and placed promptly in the dopa solution. Since the reaction is an oxidation the dish is left uncovered for free access of air.

TEMPERATURE AND TIME

The dish of dopa containing the sections is put in the incubator at 37° C for about half an hour. Then the fluid is replaced by fresh solution, which in the meantime has been kept cold in the refrigerator. For this renewal of the solution there is a reason. Some tissues are sufficiently acid to lower the pH below 7.0, in which event the fluid remains red and the cells do not oxidize dopa to melanin. They remain colorless. On the other hand, tissue that has been in formalin, especially neutralized formalin, hastens the oxidation, darkens the fluid prematurely and easily overstains. Since it is impossible to foresee the presence of these disturbing factors, and since water

buffer, obtaining a pH of 8.2. Such solutions react quickly, in about 60 minutes at 37° C. They should be inspected every 20 minutes to forestall overstaining. These hurried reactions are apt to be overstained; the slow reactions give much more delicate pictures.

A trace of acid inhibits the reaction. A trace of alkali hastens it. All glassware therefore should be scrupulously clean.

FRESH TISSUE REQUIRED

Melanoblasts: For a dopa reaction of melanoblasts the tissue must be fresh. After death or excision of the tissue from the living body, the intracellular ferment soon diffuses into the surrounding tissue and it is quickly destroyed by most fixatives and preservatives. The ideal material is a frozen section of fresh tissue made immediately after excision from the living body. However, it is difficult to cut fresh tissue neatly. In practice we follow Bloch's present custom of hardening thin slices of freshly removed tissue in 5 per cent formalin for 2 to 3 hours. After this short fixation the tissue cuts better and such experienced observers as Bloch, Miescher, Becker and Peck testify that the reaction is in no way impaired by this short stay in 5 per cent formalin. Neither the gross specimen nor the sections should be permitted to lie in water for more than a few seconds. Water and dilute alcohol extract the ferment rapidly.

In our experience, Walthard goes too far in permitting a stay of 3 days in formalin. We have made innumerable efforts to preserve tissue overnight for a dopa reaction the next day. We have tested commercial formalin (plain and neutralized with sodium hydrate), and Merck's neutral formalin, both plain and further neutralized with chalk (Cajal's practice), from 1 per cent to 100 per cent. Melanoblasts last best in 5 per cent formalin, whether neutralized or not. However, the result is always a gamble and depends on the quantity of ferment originally present in the cells. If in the fresh tissue the dopa-positive cells are black and numerous, they may still be found after 3 weeks or more in 5 per cent formalin. If in the fresh tissue the dopa-positive cells are pale, indicating little or feeble ferment, they will disappear in any formalin within 10 to 12 hours. All workers agree that the best concentration of formalin is 5 per cent. Weaker solutions extract the ferment; stronger formalins abolish the reaction quickly.

frequently to prevent overstaining. The objection to rapid reactions at high temperatures is that the fluid soon darkens from spontaneous oxidation of dopa to melanin. The dopa-melanin stains the whole section an even dark brown. In these rapid reactions it is not easy to seize the exact point where the reaction should be checked to prevent overstaining. Bloch is certainly right in insisting that the slower reactions give the more delicate pictures.

COUNTERSTAINING AND MOUNTING

The reaction finished, wash the sections in water, dehydrate, clear and mount in balsam, or counterstain in any way desired. The dopa stain is a fast black which resists all the usual reagents except hydrogen dioxide and similar oxidizing bleaches. The paradox of melanin being produced by the oxidation of dopa, and disappearing with further oxidation, is explained by its being the one colored stage in a series of oxidations, the stages before and after it being quite colorless.

The browns, blacks and grays of a correct dopa reaction form an extremely delicate picture. We dislike to obscure it with a counterstain. A dopa section, a silver stained section and a wholly unstained section mounted side by side constitute a very instructive series. Some sections of the batch may be counterstained for general topography or for special features, such as mast cells, plasma cells, or elastic fibers. As a counterstain Bloch and dermatologists generally use methyl green-pyronin. We prefer cresyl violet well differentiated with alcohol, as giving a paler ground. All counterstains take better if the dopa sections are first dehydrated, cleared, and brought back through alcohol to water.

SURGICAL PREPARATION OF THE SKIN

Through all dopa literature runs the warning that surgical preparation of the skin with chemicals, especially with iodine, inhibits the dopa reaction. If true, this would be unfortunate; for most of the skin coming into a surgical laboratory has been painted with iodine. However, the statement cannot be strictly true because the greater part of our collection, including some of our finest specimens of dopa-positive dendritic cells, consists of skin that was painted with iodine and washed with alcohol in the usual surgical way.

cannot be used to wash them out, we make it a routine practice to change the dopa. The first dopa washes out any objectionable substances and the reaction proceeds unhindered in the fresh solution. At times we have found sections of rectum and colon so acid that two changes of dopa were required before the red of the acid solution changed to the sepia brown of a correct reaction. Under these circumstances a liberal quantity of dopa solution should be used.

Having replaced the first dopa with fresh solution, the reaction is inspected every half hour. In 2 or 3 hours the fluid turns reddish, then sepia brown. The appearance of the sepia tint signals the end of the reaction. At this point a section is rinsed and examined under the microscope. In the perfect reaction the bodies of the dopa-positive cells (melanoblasts and leucocytes) are gray or black, melanin retains its natural yellowish brown color, and collagen is colorless or the palest shade of gray. If a darker stain of melanoblasts is desired, the section is returned to the dopa solution for another half hour or so. The beginner will do well to mount a section every half hour from the beginning to the end of the reaction, continuing until the solution has become black. Such a series of sections is an instructive panorama of the progress of the reaction. It will show him clearly that much of the criticism of the dopa reaction is based on overstained sections.

The time in the incubator will vary with different specimens. Of two tissues prepared alike, cut and dropped into separate dishes of dopa at the same time, one may darken more quickly than the other. The color of the section is a fair guide to the progress of the stain. A well stained section is colorless or pale gray; a pronounced smoke gray indicates overstaining. However, it is much better practice to control the reaction with the microscope.

Bloch, Walthard and European writers generally prefer a slow reaction at a lower temperature, leaving the sections in dopa for from 12 to 24 hours "at room temperature," which they state to be 18° C. In an American laboratory it is difficult to secure a constant temperature of 18° C (64° F). Our own laboratory is 23° C (73° F) in winter and from 26 to 28° C in summer. Sections left in dopa overnight are invariably found to be overstained in the morning. For this reason we use 37° C as more easily controlled.

The reaction proceeds much more quickly in the paraffin oven at 56° C. At this temperature the sections should be inspected more

again in distilled water; leave 10 minutes in saturated solution of hypo, wash in distilled water, stain the nuclei with hematoxylin and mount in balsam.

SUMMARY

A simplified technique for the dopa reaction is described and discussed in detail.

NOTE: For the interpretation of the dopa reaction with illustrations and complete literature, the reader is referred to the previous paper.⁵

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In order to test this point, in the excision of a series of scars and pigmented moles Dr. Jerome Webster kindly offered to use no skin preparation other than washing with soap and water followed by alcohol. These specimens were sectioned and placed in dopa within an hour of their excision, without contact with formalin or even with water, the sections being dropped from the knife directly into dopa solution. The results were very fine but we cannot say that they were uniformly better than in many similar specimens that had been painted with iodine and immersed in dopa with equal promptitude. As already noted in the efforts to preserve melanoblasts in formalin and with refrigeration, it is possible that strongly positive cells survive the iodine, while weakly positive cells disappear. Be that as it may, our experience indicates that the prospective investigator of the dopa reaction need not be deterred from using surgical material, even though it has been treated with iodine. We have even had good reactions from skin and mucous membranes that had been painted with picric acid, as used in the Squier Urological Clinic, to which we are indebted for some very fine specimens.

LEUCOCYTES

Myelogenous leucocytes stain more quickly than melanoblasts. To secure a delicate picture of leucocytic granules, the reaction must be checked before the melanoblasts are fully stained. As in the Schultze-Winkler reaction, leucocytes stain more uniformly in a strongly alkaline solution. Taking advantage of this principle, Bloch and Peck⁴ have recently recommended a special dopa technique for myelogenous leucocytes in blood films; it is useful for sections also.

The films are fixed in hot formol fumes for 20 minutes and immersed in 1:1000 dopa prepared with physiological salt solution. Then 0.2 cc. of 0.1 normal sodium hydrate is added for each 10 cc. of the stock dopa. The mixture turns yellow, then brown. It is left at room temperature for from 1 to 2 hours. A slide is examined microscopically every half hour. When the leucocytic granules are stained uniformly, usually in 1 to 1½ hours, the slides are washed in running water and treated like ordinary films.

Bloch and Peck have devised an ingenious method of accentuating the sharpness of the granule staining by washing the film in distilled water and immersing it in 2 per cent silver nitrate for 2 hours. Wash

In the first group of joints a thin strip of cartilage was removed from the weight-bearing surface of the medial femoral condyle and from the middle of the patellar groove. In a second group of joints a single thin fragment of articular cartilage was removed from the patellar groove, one-half of the fragment being replaced as a loose body or cartilage "joint mouse." From the third series of joints a strip of articular cartilage and subchondral bone was removed from the concavity of the patellar groove, divided, and one fragment was returned to the joint cavity as a cartilage and bone "joint mouse." In two dogs disarticulation through one knee joint was done. In these instances the synovial membranes and joint capsules were sutured over the exposed femoral articular surfaces. All operations were performed aseptically under ether anesthesia. Postoperatively the dogs were allowed the freedom of an indoor stall and an outdoor pen. The joints operated upon were not immobilized or splinted.

After varying periods of time each dog was etherized and the blood vessels of the rear extremities were perfused with 6 per cent acacia-saline solution. The perfusion was terminated by the injection of a suspension of graphite. This procedure was carried out so as to fill as many of the blood vessels and capillaries as possible with a substance that could easily be recognized both on macroscopic and microscopic examination. A 6 per cent acacia solution made up in 0.85 per cent sodium chloride solution was used as the perfusate. The graphite suspension was prepared from Hydrokollag 300, as described by Drinker and Churchill.¹ The suspension was repeatedly centrifuged until aggregates large enough to cause embolism were reduced to a minimum.

The injection of the blood vessels of the rear extremities was accomplished by means of a perfusion pump.* The following method was used. Each dog was anesthetized with ether followed by sodium veronal intravenously 25 to 35 mg. per Kg. A midline abdominal incision was made and the large vessels of the abdomen and pelvis were exposed. Loose ligatures were passed around the lower abdominal aorta and at the same level around the inferior vena cava. The sacral artery was then freed of all its branches and a wash-out cannula directed toward the aorta was inserted. The pump was ad-

* For the use of this apparatus we are indebted to Dr. C. K. Drinker. The method of operating this pump is described by Drinker, C. K., Drinker, K. R., and Lund, C. C., *Am. J. Physiol.*, 1922, 62, 1-92.

A STUDY OF THE REPAIR OF ARTICULAR CARTILAGE AND THE REACTION OF NORMAL JOINTS OF ADULT DOGS TO SURGICALLY CREATED DEFECTS OF ARTICULAR CARTILAGE, "JOINT MICE" AND PATELLAR DISPLACEMENT *

GRANVILLE A. BENNETT, M.D., AND WALTER BAUER, M.D.

WITH THE SURGICAL ASSISTANCE OF

STEPHEN J. MADDOCK, M.D.

*(From the Department of Pathology and the Surgical Research Laboratories,
Harvard Medical School, and the Medical Clinic of the
Massachusetts General Hospital, Boston, Mass.)*

A review of the literature reveals that opinion is divided as to whether or not articular cartilage is capable of regeneration. Certain workers have reported having observed regeneration of articular cartilage, yet the explanations offered regarding the manner of this regeneration are not in agreement. Because of these conflicting reports it was decided to study surgically created defects of articular cartilage in order to determine not only the ability of articular cartilage to regenerate and its manner of regeneration, but also whether or not any constant intra-articular changes could be ascribed to the existing articular cartilage defect. In certain experiments a portion of the removed cartilage was replaced in the joint from which it was removed in order to note its fate and to see if its presence resulted in any pathological changes. In a few experiments marked joint changes were associated with accidental displacement of the patella. These are reported because they seem to be of value in explaining similar changes noted by previous workers.

MATERIALS AND METHODS

The knee joints of normal, young adult dogs were used in all experiments. Each experimental procedure was carried out upon at least four joints so as to obtain for study similar lesions of four, twelve, twenty and twenty-eight weeks duration.

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depth of the patellar groove. The patella was replaced and the incision was closed in layers by continuous sutures. The skin was approximated with interrupted mattress sutures of silk and a colloid dressing applied.

The fragments of cartilage removed were measured and placed in Zenker's fluid for fixation. Subsequent histological sections showed all of these fragments of cartilage to be entirely normal. In two of the lesions the calcified zone of cartilage had been removed in small areas, together with the overlying articular cartilage. Such traumatization was apparent at operation because of slight oozing of blood from the injured subchondral blood vessels. Postoperatively, a small effusion occurred in three of the joints. In these joints, and two others showing an increase in synovial fluid, the patellae were found displaced to the medial aspect of the femoral articular surface. Such patellar displacement is worthy of emphasis since it appeared to be the important factor of sterile irritation which resulted in extensive intra-articular pathology, whereas in the joints in which the patellae remained in their normal positions, the changes observed were very slight, or absent.

Macroscopic Examination of Joints

The knee joints containing defects in articular cartilage of four, twelve and twenty weeks duration were all found to contain an excess (2 to 5 cc.) of synovial fluid which was viscid and light amber in color. Differential cell counts made on these fluids immediately postmortem showed an average of 62 per cent mononuclear phagocytes, 19 per cent lymphocytes, 17 per cent polymorphonuclear leucocytes and 2 per cent synovial cells. The patella in each instance was displaced so that it rested upon the inner side of the medial patellar ridge of the femur. The patellae showed varying degrees of atrophy and degeneration most marked in the joints with lesions of four and twelve weeks duration. In the joints least involved the cartilage had disappeared in an area of about 5 mm. in diameter, leaving subchondral bone exposed. In the most markedly altered joint the patella showed practically complete degeneration of cartilage with roughening and fragmentation of the underlying bone.

The synovial membrane in these joints showed marked villous overgrowth in the more vascular portions. On microscopic examination these villi were seen to consist of vascular connective tissue

justed so as to deliver 220 to 260 cc. per minute of the warm (37° C) perfusate at a pressure which was approximately the same as the blood pressure of the dog. As the perfusion was begun the ligature about the aorta was tied and the right side of the heart was opened. In this manner a circulation of 6 per cent acacia was substituted instantaneously for the normal circulation of the rear extremities. Perfusion was continued until all grossly detectable blood had been washed out. At that time 100 cc. of prepared graphite suspension was forced into the cannula through the wash-out opening by means of a 100 cc. syringe. Enough pressure was used to maintain a short column of graphite ahead of the perfusing fluid. As the last of the graphite was injected the sacral artery and inferior vena cava were ligated so as to prevent its escape. The rear limbs were then skinned, amputated and before immersion in 10 per cent formaldehyde solution the muscle bellies were separated sufficiently to allow ready penetration of the fixative. After the legs had become well hardened, the soft tissues were completely removed. The joints were opened, examined and photographed. Numerous blocks of tissue from the articular surface, underlying bone and synovial membrane were taken for microscopic study. The blocks of tissue containing bone were decalcified in 5 per cent nitric acid solution and embedded in celloidin. The blocks of synovial membrane were for the most part embedded in paraffin. Microscopic sections were stained routinely with hematoxylin and eosin. Occasional sections were stained by special methods for the demonstration of fibrin and collagen.

EXPERIMENT I. REPAIR OF DEFECTS IN THE HYALINE CARTILAGE OF THE WEIGHT-BEARING AND NON-WEIGHT-BEARING ARTICULAR SURFACES, AND THE REACTION OF JOINTS TO DISPLACED PATELLAE

Operation: Each knee joint was opened by a longitudinal incision just lateral to the patella. Bleeding into the joint space was carefully avoided. The patella was displaced medially and the joint was sharply flexed so as to expose the weight-bearing articular surface of the medial condyle. A small, thin piece of cartilage which averaged 4.6 by 3 by 0.5 mm. in size was removed from this area by means of a gouge. In each joint another thin strip of cartilage averaging 10 by 3.3 by 0.5 mm. in size was removed in the longitudinal axis from the

the former location where pressure and friction of opposing articular surfaces may have retarded or prevented its formation.

The most recently made defect (four weeks) in the patellar groove was covered by a slightly adherent mass of fibrin. This fibrin clot was attached to the synovial membrane at the upper margin of the articular surface by two thin, narrow adhesions (Fig. 6).

The lesions in the patellar grooves of twelve and twenty weeks duration were completely or in part covered by a vascular connective tissue which could be seen in the gross examination to extend downward from the upper margin of the articular cartilage (Fig. 2).

The remaining joint in this experiment represented lesions of a twenty-eight week period. In this joint the patella was found to be in its normal position. Clinically there was no evidence of effusion and no excess of fluid was found when the joint was opened. There were no important changes from normal in the synovial membrane or articular cartilage. The defects in cartilage were very similar in gross appearance to the freshly made defects as viewed at operation (Fig. 7).

Microscopic study of the areas where articular cartilage had been worn away by the friction of the dislocated patella revealed flat even surfaces of uncovered subchondral bone which were polished and eburnated (Fig. 8).

Microscopic Examination of the Defects in Cartilage

Histological study of the lesions of four weeks duration on the weight-bearing and non-weight-bearing surfaces of cartilage revealed them to be very dissimilar. The lesion in the femoral condyle was an empty concavity extending down to the deepest one-third of cartilage. All of the cartilage cells had disappeared from a surrounding zone of matrix for a distance approximately equal to the width of the two columns of cartilage cells. In this acellular zone the cartilage matrix stained lightly and was fibrillated. Occasional recognizable lacunae from which cells had disappeared were seen. The surrounding columns of cartilage cells converged slightly toward the base of the defect. Several enlarged rounded clusters of cartilage cells were prominent in each section at the junction of the acellular zone of cartilage matrix and the deeper normal appearing cartilage (Fig. 9). The defect in the patellar groove was filled with a fibrillar mass

centers, surrounded in each instance by a number of layers of synovial lining cells. With few exceptions such villi were but moderately infiltrated with lymphocytes and mononuclear phagocytes. There was no exudate or cellular reaction in any of the joints of a degree suggestive of bacterial infection. Another manifestation of these joint changes was that some form of pannus had grown out from the synovial membrane at the margin of the articular cartilage. Such pannus was made prominent by the intra-vascular graphite injection (Figs. 1 and 2). The defect in the patellar groove of twelve weeks duration (Fig. 2), was covered in its upper one-half by the downgrowth of blood vessels and connective tissue from the upper margin of articular cartilage. The blood vessels and capillaries were very numerous and well filled with graphite (Fig. 3). In the joint containing lesions of twenty weeks duration the entire cartilage of the patellar groove was covered by pannus.

In all joints with dislocated patellae the articular surfaces were greatly altered. Marginal proliferation of articular cartilage had occurred (Fig. 4). Such proliferative changes were very marked in the joint which had been operated upon but four weeks previously (Fig. 5). The margins of cartilage in these joints were raised, scalloped and nodular. Numerous blood vessels and capillaries had grown inward from the adjoining synovial membrane. Histological examination revealed that the elevation of the articular cartilage margin was due in large part to the formation of new subchondral bone. In contrast to these proliferative changes there were areas of degeneration and atrophy of cartilage on the inner sides of the medial patellar ridges where the patellae had rested (Fig. 6). These proliferative and degenerative changes in articular cartilage, together with the overgrowth of subchondral bone, are similar to the changes encountered in human hypertrophic arthritis.

The dimensions of the surgically created defects in cartilage which were not obscured by pannus corresponded very accurately to the dimensions of the fragments of cartilage removed. Usually the margins of these defects were slightly rounded and less distinct in outline than when first made. In most instances they appeared slightly more shallow. All of the defects on the weight-bearing surfaces of the medial femoral condyles were more sharply defined than the majority of lesions in the patellar groove. This difference was due in part to the absence of any pannus overgrowth or fibrinous deposit in

In the joint representing lesions of twelve weeks duration the upper portion of the defect in the patellar groove was covered by vascular connective tissue "pannus" which extended onto the margin of the adjacent normal articular cartilage in a thin layer (Figs. 2 and 3). This pannus was slightly attached to the underlying cartilage by occasional fibroblastic-appearing cells which extended across the line of junction. In the lower one-half of the defect the gap in cartilage was filled with newly formed tissue which was intimately fused with the original cartilage and contained no injected blood vessels or capillaries. In places no separating line between original cartilage and recently formed tissue could be distinguished because of the close similarity of the newly formed intercellular material and the original cartilage matrix. Clusters and columns of well formed cartilage cells extended across the line which marked the boundary of the defect into the newly formed tissue. It was evident in this specimen that proliferation of cartilage cells had occurred (Fig. 12). The superficial cells of the repairing tissue had the morphology of fibroblasts and merged into the superficial cartilage at the margin of the defect. Examination of serial sections through the defect in the weight-bearing surface of this joint revealed that a few small blood vessels accompanied by fibrous tissue had grown in from the perichondrium at the articular margin. A number of these blood vessels had become thrombosed. The repairing tissue in its deepest layers resembled cartilage. In it were clusters of cells within lacunae. The matrix of the newly formed and old cartilage was fused. In several areas it appeared that the newly formed cartilage was growing out from old cartilage and that actual regeneration of cartilage had taken place. This impression was gained because clusters of cartilage cells extended across the line of fusion between the newly formed tissue and original cartilage. Some of these cells could not be distinguished histologically from those in the normal cartilage. There were no demonstrable alterations in the adjoining tissues.

The reparative processes in the lesions of twenty weeks duration were somewhat different from those already described. Vascular connective tissue filled the defect in the patellar groove. In this instance, however, it spread out in a thin layer over the entire articular surface. Although the tissue deepest in the defect slightly resembled cartilage, it was not intimately fused with original cartilage and there was no histological evidence that proliferation of cartilage cells

which stained in a manner characteristic of fibrin. The presence of such a fibrin clot seems readily explainable on the basis of injured capillaries and blood vessels where the subchondral bone had been exposed and traumatized by the abnormal position of the patella on the inner side of the medial patellar ridge (Fig. 6). This mass of fibrin was seen to be undergoing avascular organization by the ingrowth of fibroblasts at the margins of the defect. In addition to the sparsely disseminated fibroblasts, the fibrin clot contained a few scattered mononuclear phagocytes and occasional polymorphonuclear leucocytes. This defect was, in its greater part, surrounded by a lightly stained zone of cartilage matrix. Differing from the lesion already described, however, the margin of this defect near the surface of cartilage was not acellular (Fig. 10). In these regions oval and fusiform-shaped cells were present. They were often surrounded by lightly stained or unstained hyaline matrix. In a number of instances such cells appeared to have been entirely separated from surrounding intracellular substance and the impression was gained that some of them were extending by direct growth into the fibrin clot. In a few areas irregular depressions in the cartilage matrix at the superficial margins of the defects were filled with these fusiform cells; some were so intimately related to the cartilage matrix as to justify the conclusion that they arose from the original cartilage cells (Fig. 11). Serial sections through the two adhesions which extended from the synovial membrane above the articular cartilage into the upper portion of the fibrinous mass revealed that fusiform-shaped cells morphologically characteristic of fibroblasts were growing through these strands of fibrin. Thus it would appear that connective tissue cells were growing into the mass of fibrin from two sources, one the original articular cartilage, the other the synovial membrane at the upper margin of the articular surface. Such fibroblastic ingrowth appeared to be the first stage in one type of repair which was always encountered when subchondral bone had been injured or whenever sufficient intra-articular change had occurred as to result in pannus formation.

A number of blood vessels well filled with graphite extended into the deeper layers of the surrounding normal articular cartilage of this joint through gaps in the calcified zone. No other abnormalities were noted in the calcified cartilage, subchondral bone or marrow spaces.

synovial villi from the vascular portion of the synovial membrane. An exceedingly vascular pannus had overgrown the patellar groove. Numerous loose, white, rounded bodies ("joint mice") were floating free within the joint (Fig. 15). These loose bodies measured from 1 to 8 mm. in greatest diameter. It was evident that they had originated in the synovial membrane at its junction with the articular cartilage where many were in process of detachment. This observation was confirmed by histological examination. Microscopically the "joint mice" were seen to consist of circular, oval, or irregularly shaped masses of tissue which had an abundant amount of hyaline intercellular material. In some portions this intercellular material showed a fibrillar background. Several layers of cells, having the morphology of fibroblasts, paralleled the surface of these bodies. In the central portion of some of them, grouping of cells into pairs and clusters had occurred so that by virtue of their morphology and arrangement they resembled cartilage cells (Fig. 16). Very few mitotic figures were found in the peripheral layers of fibroblasts, indicating that these bodies were growing with considerable rapidity although floating free within the joint space. Thrombosed blood vessels were present within occasional floating bodies, indicating as did the gross appearances, that they had taken origin in the hypertrophied villi at the articular margins. One loose body which was thin and oval in shape was histologically consistent with the original implanted fragment of cartilage, although considerable alteration in its structure had taken place. The cartilage cells were less evenly placed in the matrix than normal, a number of them were elongated, and at the periphery at one end of the fragment there was evident proliferation of fusiform cells. This fragment was entirely avascular.

All of the remaining knee joints used in this experiment, including the one which was substituted for the original twelve week experiment, were relatively normal. The patellae were normal in relation to the other structures. None of the joints contained a demonstrable excess of fluid and there was no important intra-articular pathology other than a slight hypertrophy of the synovial villi. There was no appreciable macroscopic evidence of healing of the defects in cartilage in any of these joints.

The fragments of cartilage which were placed within the joints were recovered in three specimens and verified by histological examination. One of these fragments was free within the joint. Al-

had occurred. The lesion on the weight-bearing surface of the femoral condyle was represented by a shallow depression, the deepest two-thirds of the defect having been filled with an avascular tissue which in its deepest portion was true hyaline cartilage (Fig. 13). Examination of a large number of sections through this lesion indicated more clearly than did the sections of previously described joints, that there had been proliferation of original cartilage cells (Fig. 13). Such evidence was present in the form of lengthened clusters of cells which extended across the line of fusion of the new and old matrix. These columns of cells converged toward the defect from their basal layers. Many of the individual cells had assumed elongated shapes and certain of these cells appeared immature in type.

In creating the lesions which were to represent reparative changes after twenty-eight weeks, the calcified zone of cartilage had been broken and the subchondral bone had been traumatized. In both of these lesions new bone trabeculae had been formed and proliferation of connective tissue into the defects from beneath had occurred. In the lesion on the medial condyle a small area of this repairing tissue resembled cartilage and a new layer of calcified cartilage was in process of formation (Fig. 14). In these lesions the margins of sectioned cartilage showed no evidence of repair by regeneration.

EXPERIMENT II. "JOINT MICE" COMPOSED OF HYALINE CARTILAGE: THEIR FATE AND EFFECT UPON INTRA-ARTICULAR TISSUES

Operation: The operative procedures used for these experiments were similar to those already described. A superficial strip of articular cartilage was removed from the patellar groove. Each fragment of cartilage was divided, one portion being returned to the joint as a loose body. The remaining portion was saved for histological examination.

Because of obvious displacement of the patella in the joint of the twelve week experiment, an additional joint was operated upon. A comparison of these two joints containing identical lesions of the same duration proved to be of interest. In the joint in which the patella had become displaced the knee joint was enlarged and contained about 5 cc. of viscid amber fluid. The patella was markedly atrophied and degenerated. There was a tremendous overgrowth of

margins of the defect were sharply outlined. A narrow zone of unstained cartilage matrix formed an easily recognized boundary where original cartilage had been removed. For the most part this zone of matrix was acellular; however, a few clusters of cells from the original cartilage were extending through it to lose their identity in the newly formed repairing tissue. There was no evidence of cellular proliferation at the margins of the defects in the superficial one-half of the articular surface where there was no adjoining newly formed fibrous tissue.

The defect from the joint operated upon twenty-eight weeks previous to examination showed slight reparative changes. The defect was in large part lined by unstained matrix; however, in the depth of the crater, cartilage cells had grown into this zone from beneath to form clusters of from six to twelve cells each (Fig. 18). Several of these groups of cells were enclosed in thin-walled lacunae (Fig. 19), while in other fields the surrounding deeply stained margin of the matrix had disappeared and the cells were extending into the newly formed tissue which covered the base of the defect (Figs. 20 and 21). The base of this defect, which was entirely within articular cartilage, was covered by recently formed repairing tissue about the thickness of the calcified zone of cartilage. This repairing tissue was, on the surface, morphologically characteristic of fibrous tissue (Fig. 19). However, in the deepest portion it had the histological appearances of cartilage (Fig. 20).

EXPERIMENT III. "JOINT MICE" COMPOSED OF HYALINE CARTILAGE AND SUBCHONDRAL BONE

The operative procedure for this experiment was identical with that used in the preceding one except that subchondral bone was removed to an approximate depth of 2 mm. with the overlying cartilage. The fragments were divided and one-half of each fragment was returned to the joint. Care was taken to prevent the escape of blood into the joint space by delaying closure until all oozing from the subchondral blood vessels had been controlled.

Pathological Examination: The joint representing the lesion of four weeks duration showed no prominent intra-articular changes aside from the unhealed defect in the patellar groove. However, a swelling within the joint capsule was noted. This swelling contained

though the cartilage cells were viable, there had been considerable alteration in their arrangement, and growth of cells which had the appearance of fibroblasts was seen at one end of the fragment. The other cartilage implants had been in large part surrounded by the synovial membrane of the fat pad below the patella. One of these fragments stained in a manner characteristic of viable cartilage and the majority of the cartilage cells appeared normal. There were, however, a considerable number of empty lacunae from which cartilage cells had disappeared (Fig. 17). The remaining fragment of cartilage which was identified was of the same shape and size as when implanted. In it a large number of cartilage cells had degenerated, others were shrunken and contained pyknotic nuclei and the matrix was faintly stained. None of these cartilage "mice" had become vascularized.

Histological examination of the defect in cartilage of four weeks duration showed neither evidence of cartilage regeneration nor any other type of repair. The defect had remained as an empty concavity surrounded by a lightly stained zone of matrix from which the cartilage cells had disappeared. Death of cartilage cells had likewise occurred in the superficial portion of the articular cartilage of the patellar groove at the edges of the defect. This defect extended down to but did not include any of the calcified zone of cartilage. The remainder of the femoral articular surface appeared normal in all respects.

The defect in cartilage from the joint of the twelve week experiment was almost identical in its microscopic appearance to the one already described. In this joint, however, the cartilage cells appeared entirely normal in every portion of the joint except at the immediate margin of the defect crater. A slight amount of fibrosis of the marrow tissue immediately below the lesion in cartilage had occurred.

In the joint representing the twenty week experiment the defect extended through the calcified zone of cartilage into the superficial subchondral bone. The base of the defect was covered by avascular fibrous tissue which was about twice the thickness of the calcified zone of cartilage. This fibrous tissue resembled cartilage in its deepest layers. New bone trabeculae had been built up beneath the defect and it was evident that the fibrocartilage was still being replaced by bone. A zone of calcified cartilage had begun to reform. The

Microscopic study of the defects in these joints revealed that bone had been removed by surgical means to a depth of about twice the thickness of articular cartilage. In the earliest lesion, that of four weeks duration, the defect was represented by a deep, sharply outlined depression. At the defect margins there was evidence of great osteoclastic resorption of injured bone trabeculae with practically no evidence of bone formation. The surrounding marrow spaces were filled with vascular connective tissue which was continuous with immature connective tissue that filled the deepest portion of the defect. The margins of sectioned articular cartilage were sharply defined, acellular, and there was no histological evidence of proliferation of cartilage cells (Fig. 25). The defect of twelve weeks duration was more shallow. There was less osteoclastic resorption of original bone to be seen. New bone formation was present, as was shown by the thickening of the adjoining original bone trabeculae and the presence of numerous osteoblasts. The newly formed bone merged into the fibrous tissue which in the deepest portion of the defect resembled fibrocartilage more than in the preceding joint. The surrounding marrow spaces were filled with a very vascular fibrous tissue. In this specimen the fibrous tissue which filled the defect was fused with the deepest layer of articular cartilage at the defect margins (Fig. 25). There was, however, no evidence of proliferation of cartilage cells and the margins of sectioned cartilage were covered by light staining hyaline matrix from which the cells had disappeared.

The older defects, twenty and twenty-eight weeks duration, in this experiment had become more shallow since the greater portion of the concavity had been filled in with bone. Such bone was composed of thick, irregularly placed trabeculae. The intertrabecular spaces were largely filled with fibrous tissue and a considerable amount of apposition of bone by osteoblastic activity was present. The superficial layer of recently formed bone merged imperceptibly into the dense connective tissue and fibrocartilage which formed the surface tissue in the defect. This fibrocartilage was fused intimately with the articular cartilage at the defect margin (Fig. 26). The only histological difference between the repairing tissue and the original cartilage was that in the recently formed tissue the cells lacked characteristic grouping into lacunar spaces and columns. After studying the above sequence of changes one is forced to conclude that this newly formed and imperfect cartilage developed through stages of

a cavity which communicated with the joint space by a small sinus tract. Within the cavity were several small fragments of tissue which appeared to be the remains of the implanted loose body. It was apparent that the false opening from the joint space was due to imperfect healing of the surgical incision.

In the joints containing lesions of twelve and twenty weeks duration, as in the preceding joint, there were no important intra-articular changes from normal accompanying the defects and "joint mice" (Fig. 22). In all of these joints the patellae were found to be in their normal positions. The defects in the patellar grooves were sharply outlined (Fig. 22).

It should be emphasized again that in preceding experiments, where no patellar displacement had occurred, no important intra-articular pathology was found (Fig. 23).

In contrast to the above joints the patella in the twenty-eight week specimen was displaced so as to overlie the inner patellar ridge. As in similar instances already described, this abnormality was accompanied by extensive proliferative changes in the synovial membrane (Fig. 24), and both proliferative and degenerative changes in the cartilage of the femoral and patellar surfaces. The defect in the joint was obscured by a mass of vascular connective tissue. The fragment of cartilage and bone which had been implanted in this joint was not found.

In the joint representing the lesion of cartilage and bone after twelve weeks, the implanted fragment was found unattached in a small concavity of the fat pad just below the patella. There had been considerable absorption of bone, all of the bone cells had degenerated, and the marrow spaces were filled with fibrous tissue which resembled cartilage. There was no evidence of any bone formation, neither was there evidence of proliferation of endosteal cells. The hyaline cartilage of the fragment had remained viable in its entirety. It showed no regressive changes in either cells or matrix. This entire loose body was surrounded by a narrow layer of proliferating fibroblasts.

The loose body in the joint operated upon twenty weeks previously was partially surrounded by synovial membrane. The bony portion of this fragment had been absorbed, whereas the cartilage matrix was well preserved. The cartilage cells in the greater part of the fragment had maintained a normal appearance.

at the margins of the cartilage. All of the sections showed superficial cartilage depressions and areas in which the cartilage cells had degenerated, leaving lightly stained and slightly fibrillated cartilage matrix. In the midzone of articular cartilage many of the cartilage cells had become fusiform in shape and occurred singly or in clusters. This finding serves to emphasize the fact that mature cartilage cells may acquire, under appropriate stimulus, the morphology of fibroblasts.

DISCUSSION

Although numerous workers have studied the repair of defects made in hyaline cartilage of articular surfaces, there has been no substantial agreement of opinion regarding the ability of cartilage to regenerate, or by what method repair of cartilage occurs. It is probable that the existing confusion is due to the facts that in most publications no clear differentiation has been made between repair by proliferation of connective tissue from neighboring tissues and independent regeneration of cartilage; that the repair of lesions of cartilage with injury to subchondral bone have not been separated from the repair of lesions made entirely within articular cartilage; and that much of the work from which deductions have been drawn was done before the time when the importance of strict asepsis in joint operations was realized.

Since an inclusive review of the literature pertinent to the regeneration of hyaline cartilage has been recently recorded by Shands,² references to previous work will be minimized in this report. One may divide the opinions of earlier authors into three groups: (1) those who believe that independent regeneration of adult articular cartilage does occur; (2) those who insist that cartilage does not have the ability to regenerate, and (3) those who believe that regeneration occurs through proliferation of fibroblasts with subsequent metaplasia into cartilage.

1. Seggel³ reported that within twenty-four and forty-eight hours after a defect in cartilage had been made, the cartilage cells directly adjacent to the defect became swollen, that small cartilage islands were formed mostly in the center of the defect and that after twelve days mitoses were found. He noted that infection checked this reaction and that it was less marked in older animals. It was also observed by this author that defects near ligament or membrane attachments became covered by pannus and that centrally

metaplasia from the typical fibrous tissue which originally filled the defect (Fig. 25). Such fibrous tissue probably took origin in the connective tissue of the marrow spaces in the subchondral bone.

EXPERIMENT IV. CHANGES IN ARTICULAR CARTILAGE ASSOCIATED WITH THE REMOVAL OF OPPOSING ARTICULAR SURFACES

After unsuccessful attempts had been made to maintain separation of articular cartilage surfaces within unopened joints, disarticulation with careful closure of the synovial membrane and joint capsule over the exposed femoral articular surfaces was resorted to in an attempt to obtain some information as to the importance of apposition of cartilage surfaces in maintaining normal nutrition. The patellae and patellar ligaments were utilized in covering the denuded articular ends. The approximation of the margins of the flaps to reform a synovial-lined space proved fairly satisfactory. Specimens of twelve and twenty-eight weeks duration were obtained for study by this method. There was no material difference either in type or degree of the changes from normal which occurred in these specimens.

Pathological Examination: When the synovial membrane was incised at the margins of the articular cartilage, fine "cobweb" and coarse adhesions were encountered. These adhesions were present in several areas although there were numerous small synovial spaces remaining. The uncovered surfaces of articular cartilage were non-glistening, gray in color and showed numerous areas of partial atrophy or complete degeneration.

When gross sections of cartilage and subchondral bone were made it was noted that the cartilage was very thin, even in the least changed areas, and that there was marked atrophy of the subchondral bone.

Microscopic examination revealed that very marked thinning and decalcification of the subchondral bone trabeculae had occurred. The articular cartilage was everywhere thinned out. In places it had completely degenerated. In a number of areas the calcified zone of cartilage was much thinner than normal. In some of the sections the surface of articular cartilage was covered by a thin layer (five to ten cells deep) of fibroblasts which could be traced to the perichondrium

cartilage existed at the margins of the articular surfaces as compared to the central areas. He explained this difference on the basis of better nutrition and the presence of perichondrium in the former location. However, no clear distinction between the repair of those lesions in which subchondral bone had been injured and those in which only cartilage had been traumatized was made. More recently Shands,² after studying the repair of lesions in articular cartilage in dogs, came to the conclusion that cartilage did not regenerate in less than four weeks and that when regeneration did occur it progressed through stages of fibrin formation, granulation tissue, fibrous tissue and transformation of fibrous tissue into cartilage. He was unable to demonstrate any difference in the regenerative powers of cartilage in the various areas of the articular surfaces. Key¹² and Ito¹³ observed that repair of defects in hyaline cartilage and subchondral bone occurred by the proliferation of fibrous tissue from the marrow spaces, with subsequent transformation of the fibrous tissue into cartilage. Attention was called to the observation that the injured surfaces of both bone and cartilage die and that repair occurs through the proliferation of the osteogenic cells lining the marrow spaces.¹²

The present series of experiments indicate that adult articular cartilage does have a limited ability to repair aseptic lesions within its substance by independent regeneration of cartilage. The powers of such regeneration, however, are feeble and not always demonstrable. The greatest regenerative activity was noted in defects on the weight-bearing surfaces of the femoral condyles, whereas a lesser proliferative activity was found in the non-weight-bearing surface of the patellar groove. It was in the latter location that no reparative reaction was seen in two joints where the lesions had been present for periods of four and twelve weeks. A satisfactory explanation of this complete absence of regeneration in the two lesions described is not possible. The fact that none did occur, however, serves to emphasize the feeble ability of cartilage cells to proliferate, particularly the cartilage cells which are most distant from the perichondrium at the articular margins. It is only fair to point out the possibility that the animals in which no repair occurred may have been older than the others, since the exact ages of the dogs could not be ascertained. Only adult dogs* showing no evidences of advanced age were se-

* The repair of articular cartilage in young dogs before epiphyseal union has occurred will be commented upon in a subsequent report.

located shallow defects and linear incisions showed no reparative reaction after long periods of time. Fasoli⁴ described degenerative changes in surrounding cartilage cells and matrix immediately following injury. At a later time he noted proliferative changes in cartilage cells at the margins of the defects with division of cartilage cells by mitosis after nine days. He described slow, progressive, proliferative changes until complete repair had occurred. Even after six months time he found doubtful evidences of continued regeneration around the defect margins.

2. Haebler⁵ concluded from his experiments that defects in articular cartilage which did not include subchondral bone showed no evidence of healing from the borders of the defects within 304 days. He further concluded that when connective tissue or fibrocartilage was found filling the injured cartilage, serial sections would reveal that subchondral bone had been injured in some small area. It was also the opinion of Geis⁶ that clean aseptic wounds in cartilage do not heal and that cartilage does not possess the power of regeneration. Geis, however, did find that in the presence of infection healing of cartilage occurred so as to leave little or no evidence of the defect. The ability of hyaline cartilage to regenerate in adult mammals is denied by Maximow and Bloom.⁷ They state that wounds repair by the ingrowth of connective tissue from the perichondrium or the nearest fascia and that the failure of independent regeneration of cartilage is due to the inability of mature mammalian cartilage cells to divide mitotically. In tangentially placed superficial wounds of cartilage which did not extend into subchondral bone, Ciociola⁸ observed scarcely any reaction. He did observe the repair of wounds in cartilage which extended into subchondral bone. Such repair occurred by connective tissue proliferation and transformation into hyaline cartilage.

3. Healing of wounds in articular cartilage, by the proliferation of fibrous tissue from one of a number of sources, has been described by several authors. Redfern⁹ in 1851 stated that wounds in articular cartilage heal perfectly by fibrous tissue, which he believed to arise from the intercellular substance and cells of the articular cartilage. Gurlt¹⁰ concluded that defects in cartilage are repaired by a fibrous, and at times cartilage-like tissue, but that it is never completely replaced by cartilage and true regeneration of cartilage does not occur. Fisher¹¹ reported that greater regenerative ability of

synovial cells becoming implanted in such a mass of fibrin, growing as fibroblasts and thus taking part in the organizing process. The fibrin which formed within this and other joints probably resulted from injury to capillaries and blood vessels where cartilage and subchondral bone were worn away by the displaced patellae. Through a continued connective tissue growth and proliferation of vascular endothelium from the articular margins, pannus was eventually formed. The defects, and later the surface of cartilage, became covered by vascular connective tissue in the non-weight-bearing surfaces of the joint. In the defects, the deepest layer of pannus later became transformed into tissue that histologically resembled fibrocartilage. In one lesion in the patellar groove independent repair by proliferation of cartilage cells was apparent in the lower one-half of the defect, whereas in the upper one-half the repairing tissue was being absorbed in the pannus which was growing downward from the synovial membrane at the upper margin of the articular surface.

A third type of repair took place in those lesions which extended into subchondral bone. In such instances a proliferation of connective tissue from the marrow spaces occurred. Proliferating fibroblasts, accompanied by fairly numerous blood vessels, filled the deepest portion of the concavity of the defect and the surrounding marrow spaces. In the older lesions it was noted that a great deal of intercellular substance had been formed by the fibroblasts. In the deepest portions of the defects the newly formed tissue had been transformed into bone. The new bone merged into an intermediate layer of dense, rather avascular connective tissue, while on the surface the repairing tissue had acquired the morphological appearance of imperfect hyaline cartilage. The original bone trabeculae about the margins of the defects had been widened by osteoblastic activity and the marrow spaces had maintained a richer blood supply than normal. In occasional specimens a new zone of calcified cartilage had partially reformed and extended into the repairing tissue from the calcified zone of cartilage at the margin of the defect. It was not until periods of twenty or twenty-eight weeks had elapsed that the defect crater had in large part been filled. At that stage the surface layer of newly formed tissue was avascular, resembling cartilage in the amount and staining quality of its intercellular substance and in the morphology of its cells. Although the matrix of the new cartilage was fused with the matrix of the original cartilage, the new cells

lected for use. When proliferative changes do occur in defects in cartilage, one finds a convergence of cell columns toward the base of the defect with the formation of superficial clusters of cartilage cells to indicate that original cartilage cells have multiplied within lacunae. No indication as to the manner of division of these cells was obtained from these experiments, although it should be noted that mitotic figures were observed by other workers after nine⁴ and twelve days.³ No lesion in the present series was examined before a duration of four weeks. The sequence of changes which appeared to have occurred were the projection of such clusters of cells into the acellular zone of cartilage matrix at the margin of the defect, with disruption of the lacunar margins and the spread of cartilage cells over the surface of the defect. In several instances a continuation of cartilage cell columns across the line of junction of the new and original tissue could be traced. Ultimately the newly formed tissue within the defect developed, by virtue of the amount and staining quality of its intercellular substance and the morphology of its cells, a distinct resemblance to hyaline cartilage. Perfect hyaline cartilage, however, was not reformed in these lesions.

A different form of repair occurred in the defects of the patellar groove in the joints where the patellae had become displaced. In these instances vascular connective tissue (pannus) spread over the defects from the articular margins. The earliest changes of this sort were observed in a lesion of four weeks duration. The defect was filled with a mass of fibrin into which fibroblasts were growing from the superficial levels of articular cartilage at the margins of the defect and from the synovial membrane at the upper margin of the articular surface. A careful histological study of the articular cartilage at the immediate margin of the defect revealed that lightly stained or unstained cartilage matrix surrounded scattered cartilage cells. A number of these cells in each section appeared to have been liberated entirely from hyaline matrix and the impression was gained that some of them were growing into the fibrin clot and were therefore in part responsible for the early avascular organization which was occurring. Cartilage cells have been grown in tissue culture¹⁴ and it is not illogical to assume that a similar type of growth may occur within joints. The great variety of ways in which mesenchymal cells may differentiate or dedifferentiate because of location and function, causes one to consider also the possibility of detached

which the patella had become permanently displaced following the displacement at operation. Such changes did not occur in any of the joints in this series where the patella remained in its normal position. The occurrence of spontaneous and progressive defects in articular cartilage and subchondral bone in bovine joints¹⁶ without other important intra-articular changes is further evidence that the defects themselves are not a cause of other important joint changes. Associated with the patellar dislocation, each joint of the present series showed marked hypertrophy of the synovial villi and marked marginal "lipping" of cartilage due to proliferation of the subchondral bone. Atrophy and degeneration of cartilage occurred where the patella had moved about in its abnormal location, and polishing with eburnation of the uncovered bone followed the degeneration of cartilage. In each of these joints, pannus, which was made prominent by the intravascular injection of graphite ink, developed on the non-weight-bearing surfaces of cartilage. It is a noteworthy fact that pannus did not extend over the weight-bearing surfaces of the condyles in the joints in which it had formed in other areas. This fact indicates that weight-bearing and motion of the opposing articular surfaces may retard or prevent the formation of pannus. "Joint mice" developing from hypertrophied and detached synovial villi occurred in most of these greatly altered joints.

The atrophy and degeneration of cartilage with pannus overgrowth that occurred in joints in which the femoral articular surfaces were removed from contact with other cartilage by amputation through the knee joint was similar to that observed by others.^{5, 11} These findings suggest that the apposition of one articular surface with another is probably important in the maintenance of normal hyaline cartilage.

SUMMARY

1. Studies concerning the repair of surgical defects made in hyaline cartilage of normal adult dog joints, the joint reaction to loose bodies of cartilage and cartilage with attached bone, and the joint reaction to displaced patellae are reported. Each type of lesion was examined after periods of four, twelve, twenty and twenty-eight weeks duration.
2. Some form of repair occurred in seven of the nine defects which were made entirely within the articular cartilage of the weight-bear-

did not acquire the usual distribution in columns such as is characteristic of normal articular cartilage.

The fragments of articular cartilage "joint mice" which had been returned to the joints from which they were removed were found to contain a few empty lacunae from which cells had disappeared. The majority of cells, however, were viable and the greater part of the matrix stained with normal intensity. The entire fragments were found to be of essentially the same shape as when implanted. In most instances they had been surrounded by vascular connective tissue of the synovial membrane and thus removed from the joint space. The tendency for removal of fragments of cartilage from the articular space was noted by Ito¹³ in experimenting with rats. He believed that the small size of the joints in his experiments may have been an important factor in causing their removal. In the present experiments, the bone of the cartilage and bone implants had been destroyed or was in process of being removed. In these same fragments the cartilage had not undergone necrosis, and in one instance showed no evidence of any retrograde change. It is a noteworthy fact that this fragment had remained entirely free within the joint for a period of twelve weeks, in contrast to the less well preserved fragments of cartilage which had been removed from the joints. Harbin and Moritz¹⁵ described the survival of collodion-encased fragments of articular cartilage in knee joints for as long as thirty-two days.

The findings from the present series of experiments indicate that neither the presence of surgically created defects within cartilage or cartilage and bone, nor the presence of loose bodies of cartilage or cartilage with attached bone are, in themselves, a cause of important associated intra-articular pathology. Such an observation is not in agreement with the conclusions of Key¹² who reported inconstant, but at times marked joint changes of the hypertrophic type in rabbits from which he had removed small pieces of cartilage and underlying bone. He was unable to explain the variability of the pathological changes which followed these similar operations, although he was of the belief that they were due to the presence of the surgically made defects. Haebler⁵ associated arthritic changes in experimental joints with displaced patellae. In the present group of experiments important intra-articular changes of a type similar to the changes in hypertrophic arthritis of man were encountered in every joint in

suggest the importance of the apposition and weight-bearing of adjoining articular surfaces in maintaining the proper nutrition of hyaline cartilage.

9. The application of a method of capillary and blood vessel injection with a substance, which is easily recognized on macroscopic and microscopic examination, is described.

ing and non-weight-bearing articular surfaces. The two exceptions were represented by lesions in the patellar groove of four and twelve weeks duration.

3. In the defects which extended into subchondral bone and in those defects where pannus, accompanying displaced patellae, covered the defects the reparative changes passed through stages of fibrous tissue and fibrocartilage to the formation of an imperfect form of hyaline cartilage. The fibrous tissue originated in the connective tissue of the bone marrow, in the marginal synovial membrane and apparently, in some instances, from articular cartilage cells.

4. Histological evidence of repair of cartilage by proliferation of cartilage cells was present in four of the six defects which were entirely within cartilage and not covered by pannus. Such proliferation was most marked in the lesions made in the weight-bearing surface of the femoral condyle. In no instance was repair complete or perfect within the twenty-eight week period. In the majority of lesions the repairing tissue filled but a small portion of the defect crater.

5. In these experiments marked intra-articular changes similar to those of human hypertrophic arthritis occurred in every joint in which the patella became displaced. Such joints showed pannus formation, hypertrophied synovial villi, "joint mice" formation and proliferative and degenerative changes in the articular cartilage and subchondral bone at the articular margins.

6. The presence of small defects in cartilage, or defects which extended into subchondral bone, was not a cause of important joint pathology in these experiments.

7. Cartilage and bone and cartilage fragments returned to the joints from which they were removed did not produce any significant intra-articular changes. The bone of the bone and cartilage fragments had been resorbed or was in the process of resorption, whereas the cartilage had remained viable in large measure in all instances. In the majority of specimens the implanted loose body had been surrounded by connective tissue and thus removed from the intra-articular space.

8. Extensive atrophy of cartilage and pannus formation over the surface of cartilage occurred within a twelve weeks period, when disarticulation through the knee joint was performed. These findings

DESCRIPTION OF PLATES

PLATE 88

FIG. 1. A gross photograph of the articular surface of the femur, showing pannus at the synovial margin of the intercondyloid notch and at the lateral margin of the patellar surface. The blood vessels are filled with orange ink. The surgically made defect of four weeks duration is visible on medial condyle. $\times 2.5$.

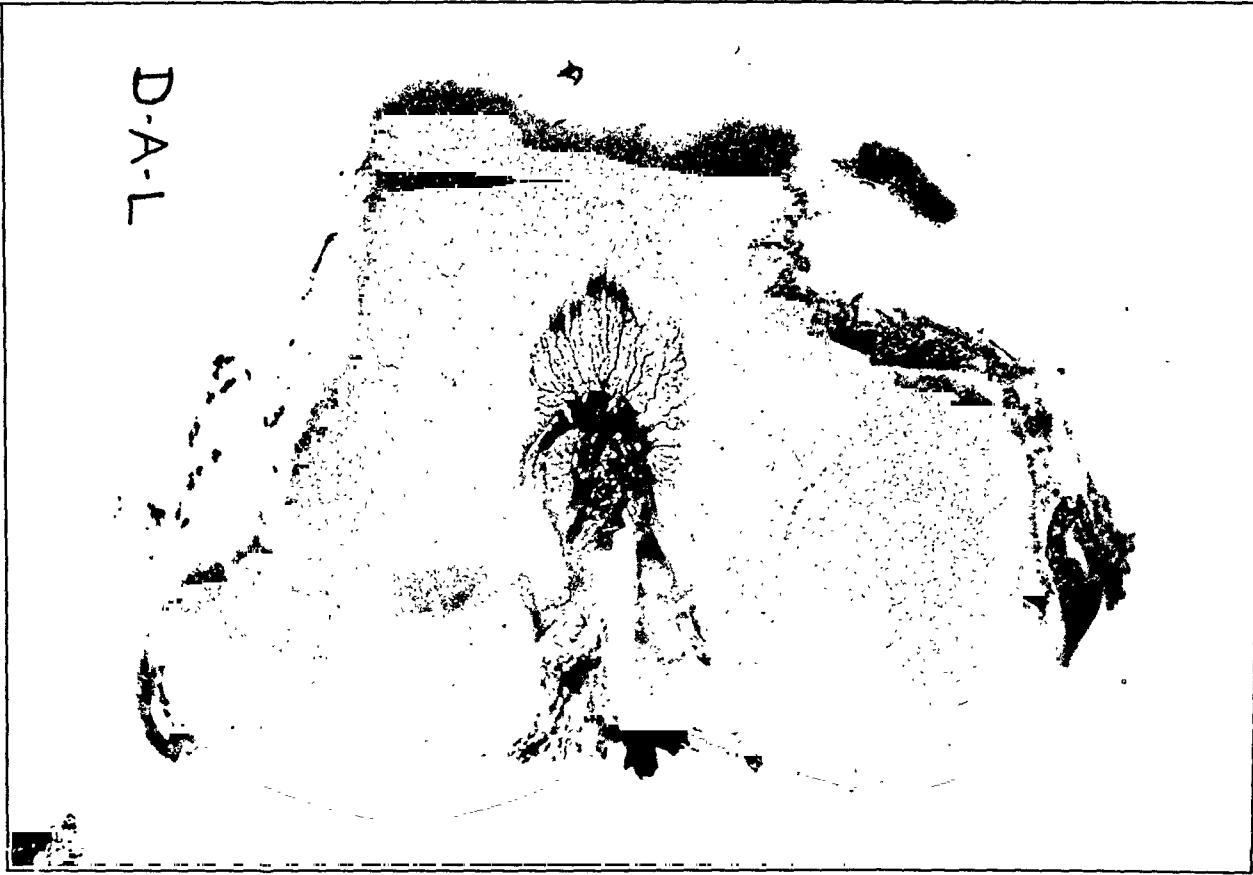
FIG. 2. An anterior view of the articular end of the femur showing surgical defects of twelve weeks duration. Associated with displacement of patella the articular surface has become widened and elevated at the margins. Note the extension of pannus from the upper margin of cartilage on the upper one-half of the defect in the patellar groove. $\times 2.5$.

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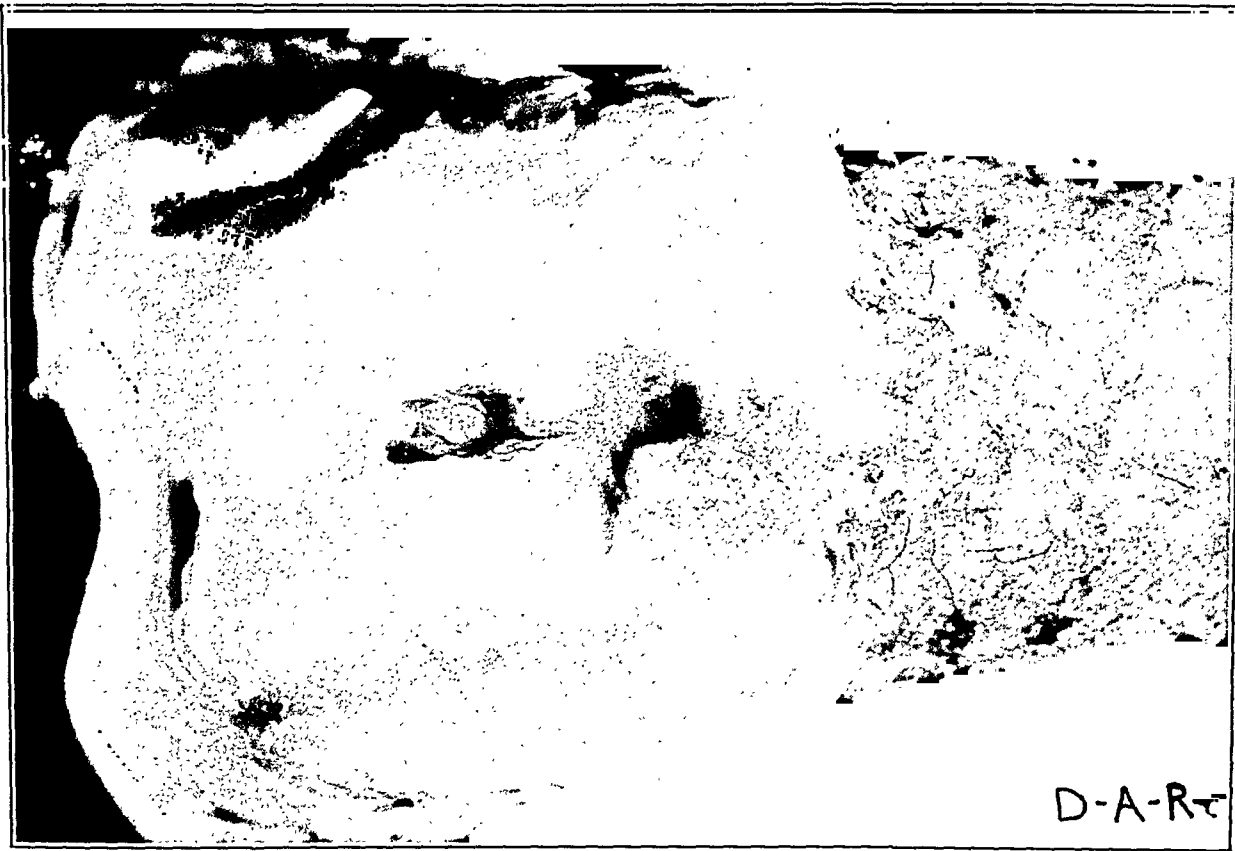
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PLATE 89

- FIG. 3. A photomicrograph which shows the changes in one-half of the twelve weeks old defect in the patellar groove. Note the numerous graphite-filled blood vessels in the pannus, the formation of fibrocartilage in the deepest layer of repairing tissue and the sharply outlined and acellular margin of the defect. The section was made through the upper one-half of the lesion into which blood vessels had extended (Fig. 2). $\times 76.5$.
- FIG. 4. Proliferative changes in articular cartilage of the hypertrophic type are illustrated in this photograph of a joint which contained defects of twelve weeks duration. The patella was displaced during the entire twelve week period. $\times 2.5$.



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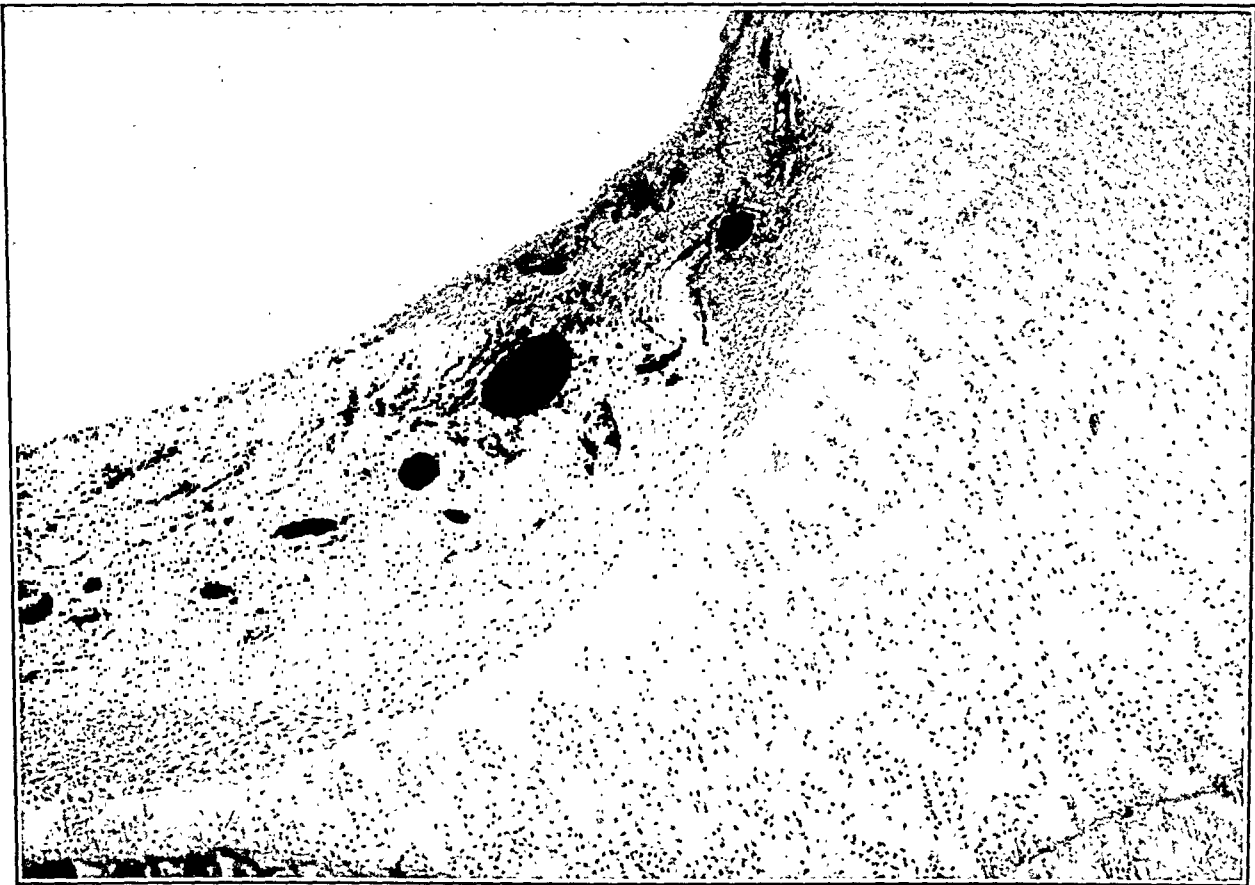
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PLATE 90

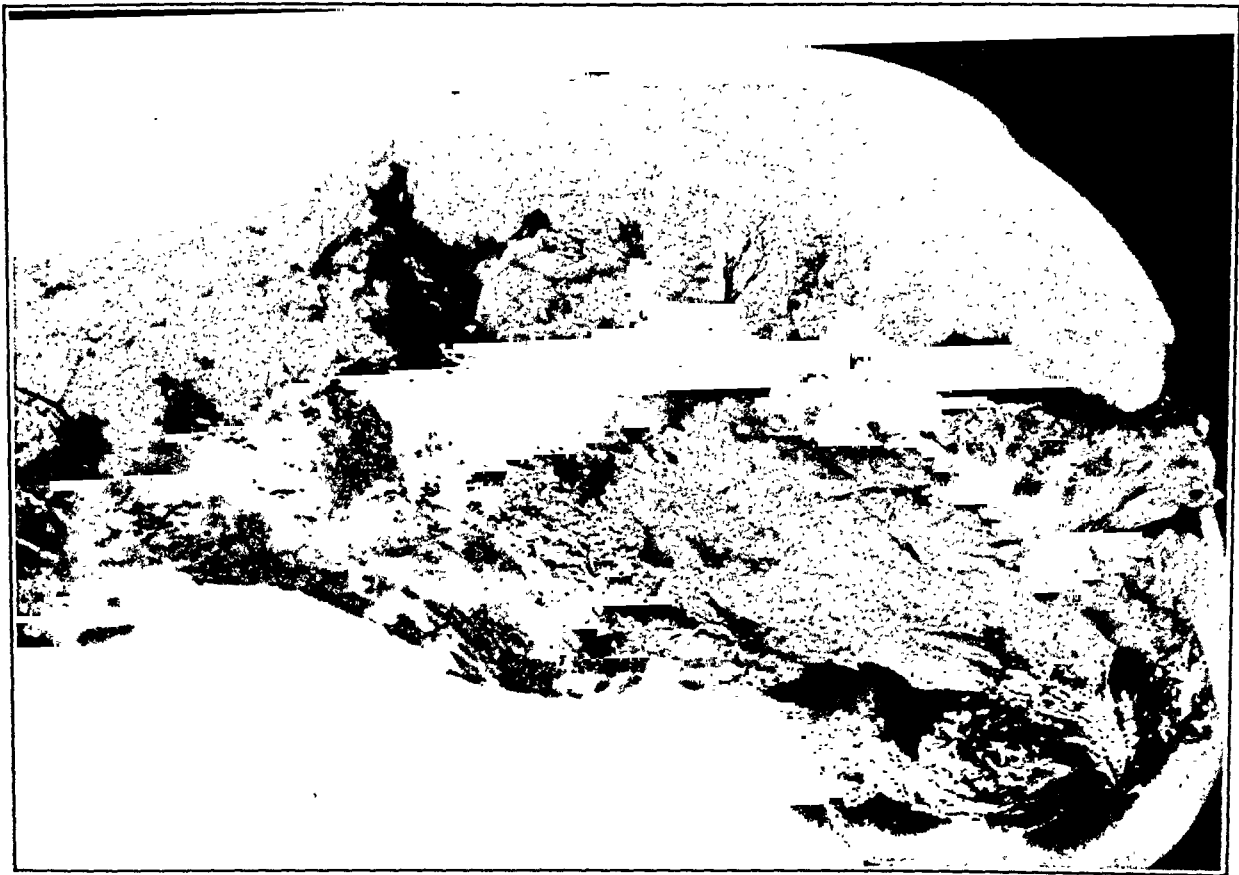
FIG. 6. A lateral view of the femoral end of a joint operated upon four weeks earlier. The patella became displaced and, as was invariably the rule, proliferative and degenerative changes in articular cartilage occurred. Note marginal proliferation, elevation and vascularization of cartilage. $\times 2.5$.

FIG. 6. A photograph of natural size, showing atrophy and degeneration of articular cartilage on the medial side of the joint where the patella had rested. The defect in the patellar groove is covered over by an avascular and partially organized fibrin clot which is attached to the synovial membrane at the upper margin of the articular surface by two adhesions.

FIG. 7. A photograph of natural size, showing the sharply outlined surgical defects in the cartilage of the patellar groove and femoral condyle after twenty-eight weeks duration. The patella was not displaced and the joint remained essentially normal.



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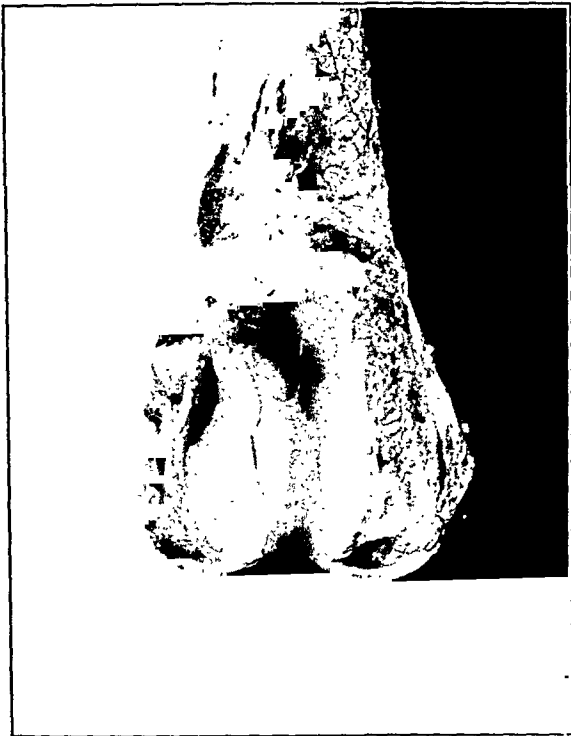
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PLATE 91

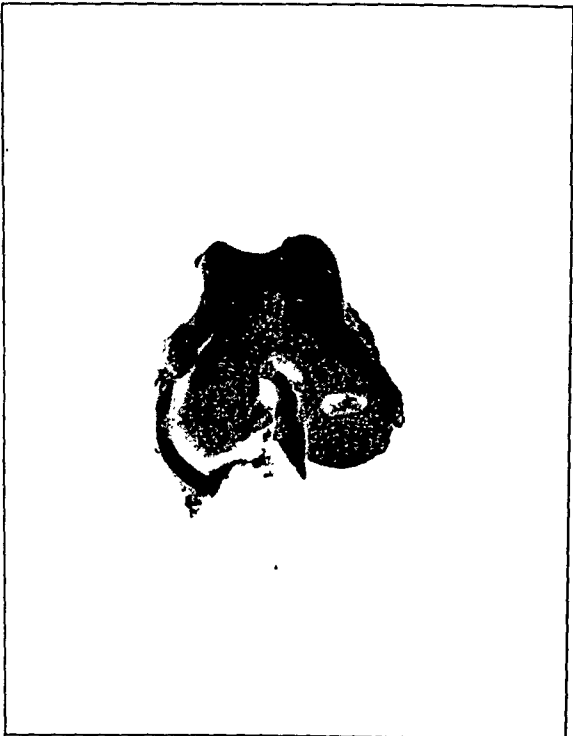
- Fig. 8. A photomicrograph showing eburnation and polishing of bone where the articular cartilage has been worn away during a four weeks period by the dislocated patella. $\times 76.5$.
- Fig. 9. A section through the entire defect in the cartilage of the weight-bearing condyle is shown in this photomicrograph. The lesion was present for four weeks. Note the acellular margin of the defect in the deepest portion, the converging columns of cartilage cells and the formation of new cartilage in the superficial one-half of the defect crater. $\times 76.5$.



5



6



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PLATE 92

- FIG. 10. A camera lucida drawing showing the margin of a defect of four weeks duration in the patellar groove. The defect is filled with fibrin undergoing organization. Note apparent proliferation of fibroblasts from the cells in the superficial layers of the original articular cartilage. $\times 170$.
- FIG. 11. A camera lucida drawing of the inset in Fig. 10. $\times 420$.



8



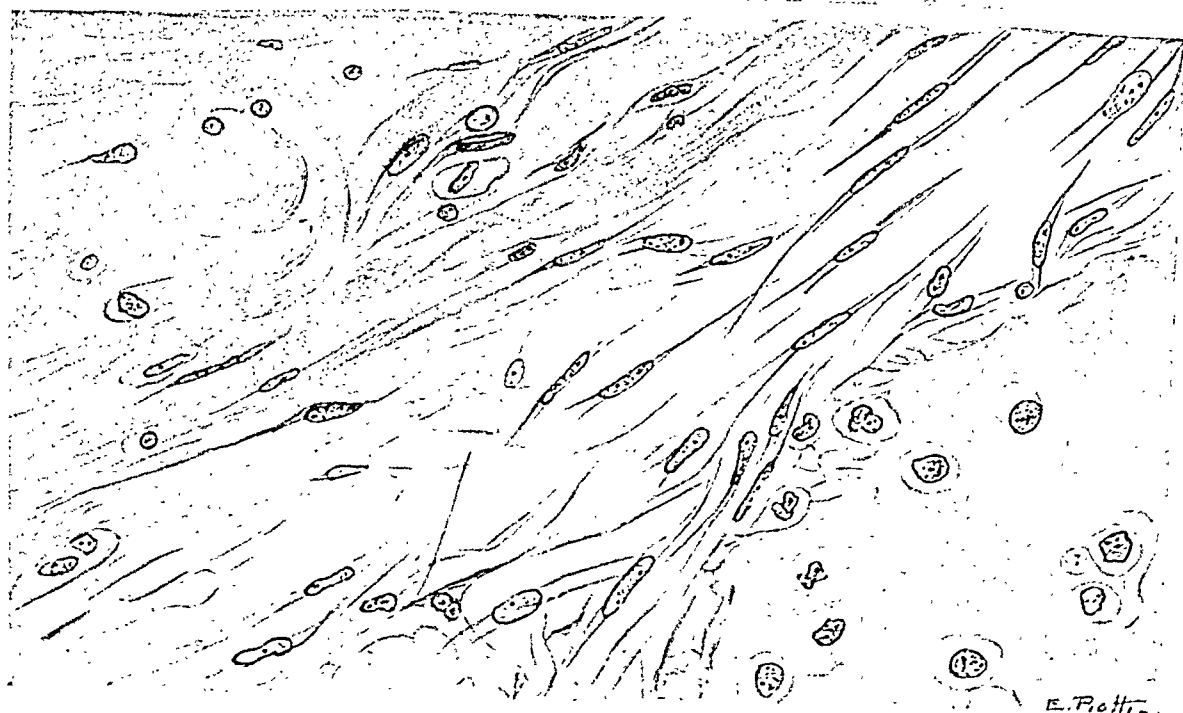
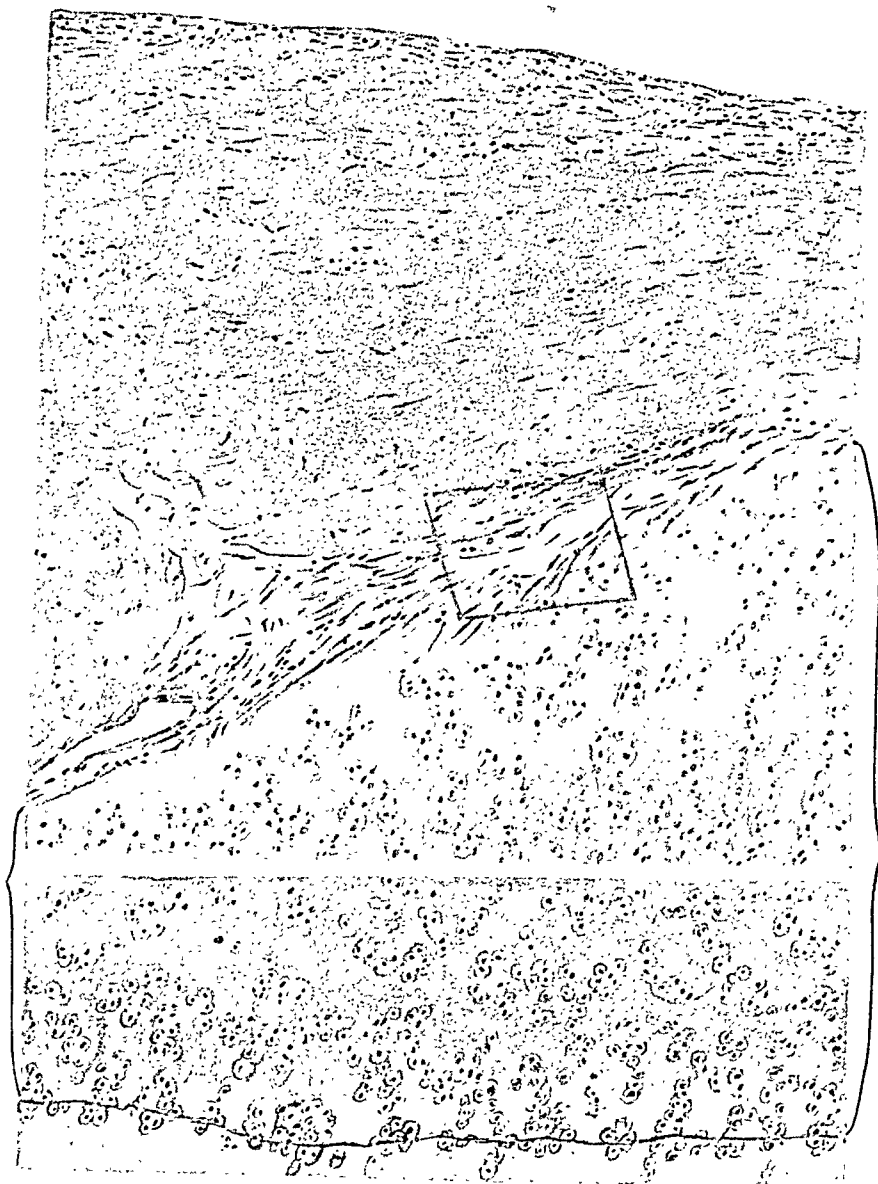
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PLATE 93

FIG. 12. One-half of the defect of twelve weeks duration in the cartilage of the patellar groove is shown in this photomicrograph. Note extension of clusters of cartilage cells across the boundary between the original cartilage and recently formed tissue filling the defect. Near the surface the repairing tissue appears to be fibrous tissue; however, in the deeper layers, it resembles hyaline cartilage. $\times 68.5$.

FIG. 13. Proliferation of cartilage cells into the defect crater is shown clearly in this photomicrograph of a section from a twenty weeks old defect in the cartilage of the femoral condyle. $\times 76.5$.

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E. P. Rott

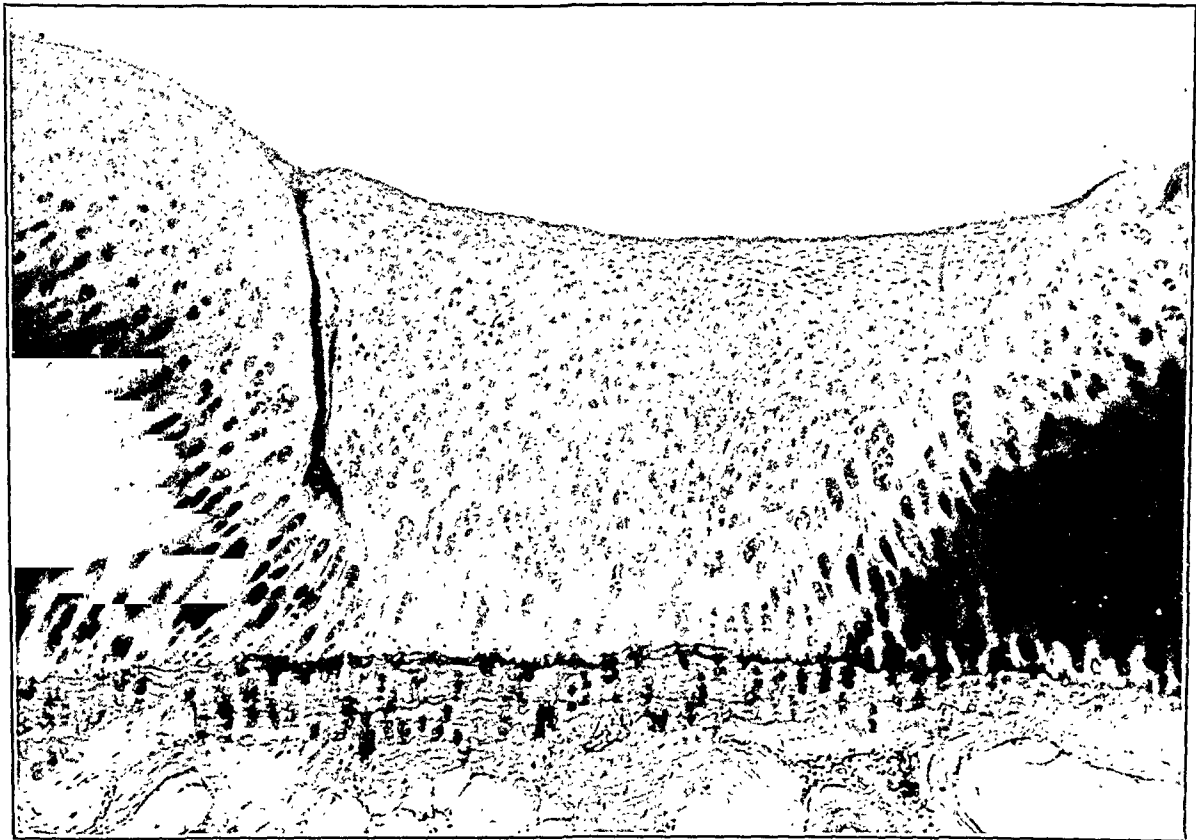
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PLATE 94

- Fig. 14. A photomicrograph showing the repair in a defect of twenty-eight days duration which extended into subchondral bone. The defect is filled with fibrous tissue, fibrocartilage and imperfect hyaline cartilage. A new zone of calcified cartilage has partially reformed. Note the acellularity of the matrix of original cartilage at the margin of the defect. $\times 76.5$.
- Fig. 15. A gross photograph of natural size showing panus covering the surface of the patellar groove and the "joint mice" which formed within the joint. Note the widened and uneven articular surface. These changes which accompanied dislocation of the patella occurred within a period of twelve weeks.



12



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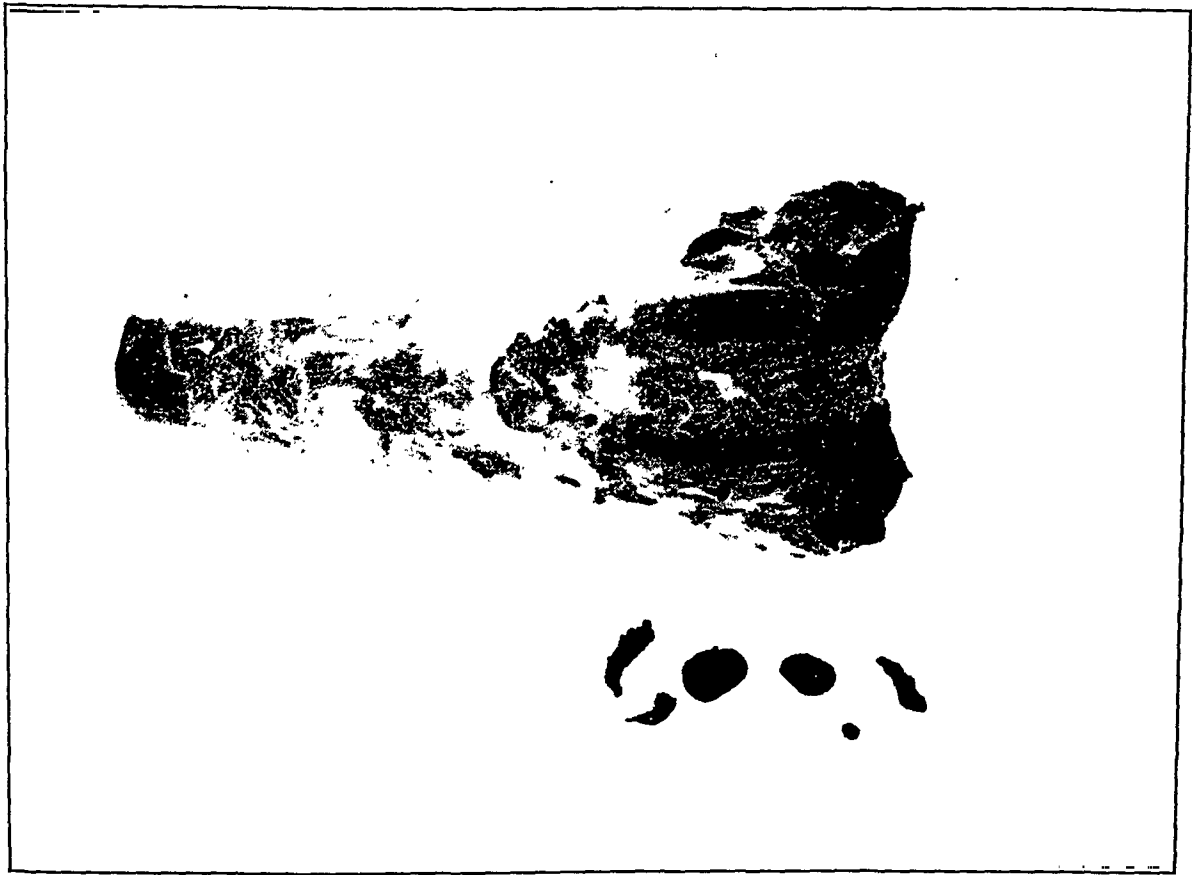
PLATE 95

FIG. 16. A photomicrograph showing the structure of the largest "joint mouse" illustrated in Fig. 15. $\times 76.5$.

FIG. 17. A photomicrograph which illustrates how implanted fragments of articular cartilage often were surrounded by the vascular connective tissue of the synovial membrane. The fragment of cartilage is in large part viable after a period of twelve weeks within the joint. $\times 76.5$.



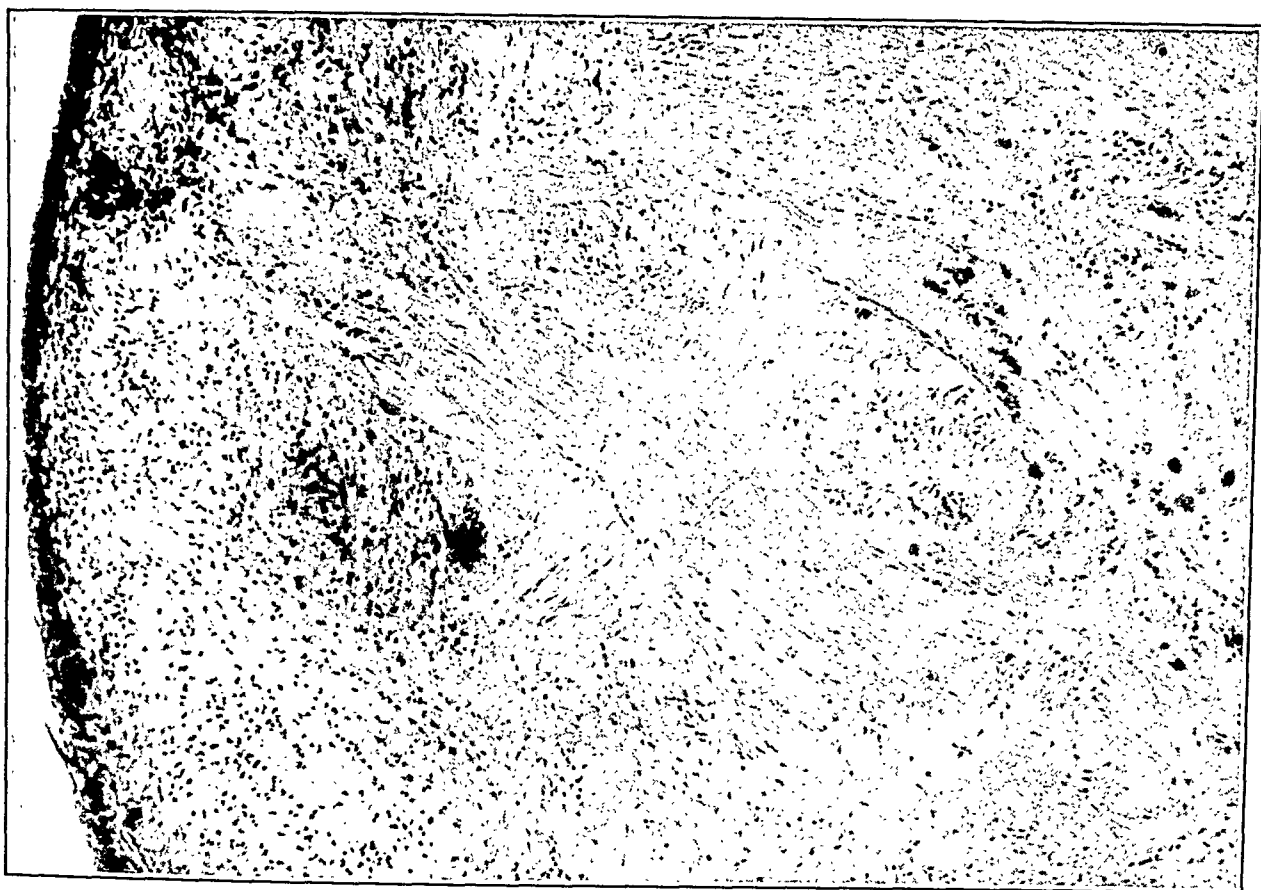
14



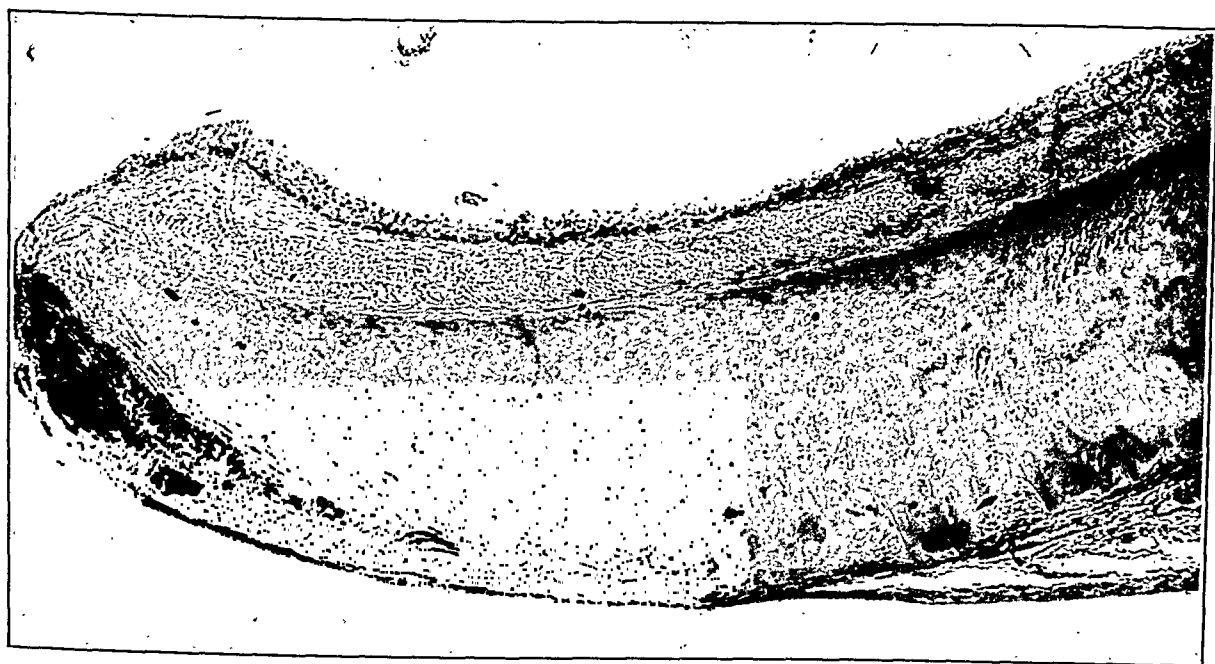
15

PLATE 96

FIGS. 18, 19, 20, 21. Camera lucida drawings of the reparative changes seen in a defect in the patellar groove after a period of twenty-eight weeks. Note the clusters of cartilage cells within lacunar spaces, the disappearance of some of the lacunar margins and the extension of the cartilage cells from the original hyaline cartilage into recently formed tissue which partially filled the defect crater. Obviously multiplication of cartilage cells within lacunar spaces had occurred although no mitotic figures were found. Fig. 19, $\times 420$; Figs. 18, 20, and 21, $\times 630$.



16



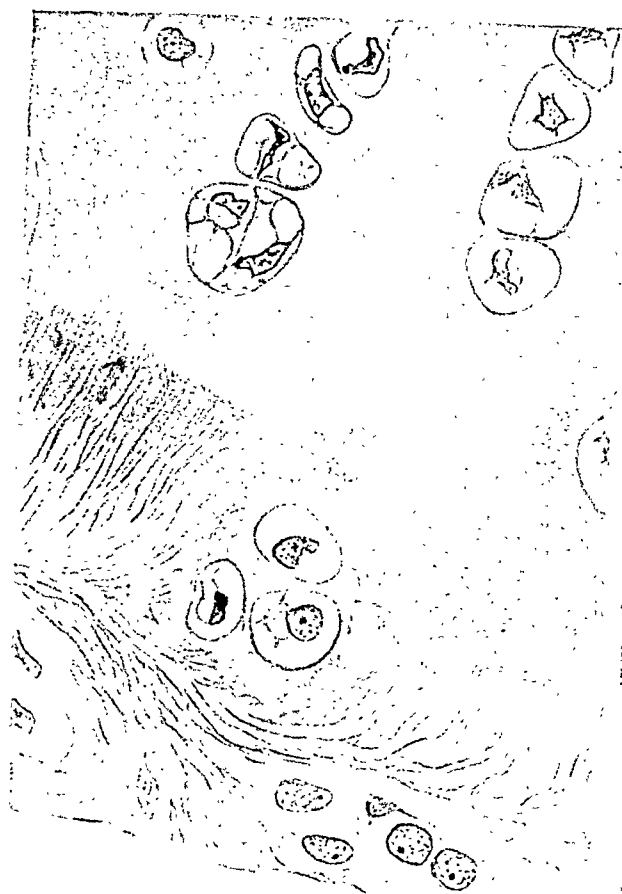
17

PLATE 97

FIG. 22. Gross photograph (natural size) showing a defect in cartilage and subchondral bone after a period of twelve weeks. No important intra-articular changes had occurred.

FIG. 23. A natural size photograph of the right and left joints showing no important difference between them except for a surgically made defect in the cartilage of the patellar groove of the right joint. The right knee joint was operated upon twelve weeks before, the left joint served as a control.

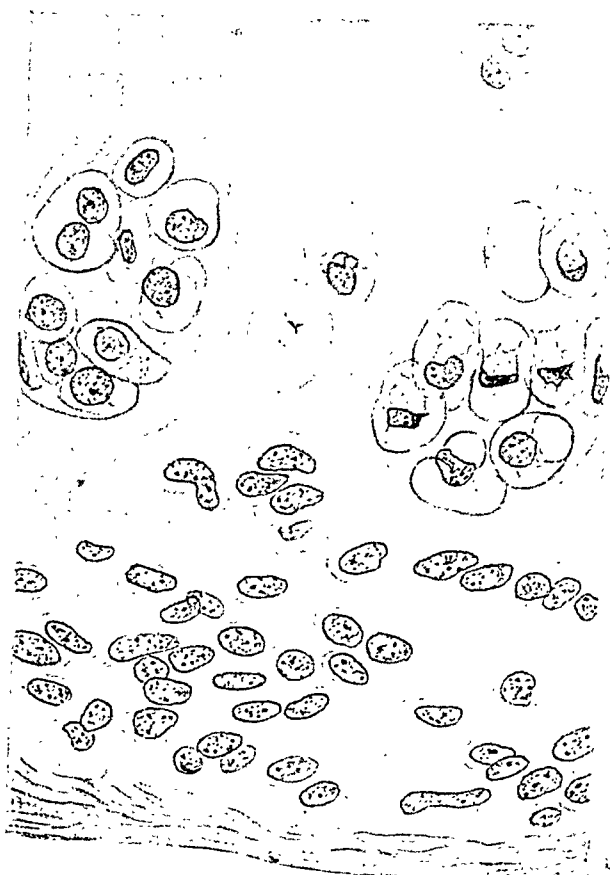
FIG. 24. A gross photograph which illustrates the marked villous overgrowth of the synovial membrane which occurred in the joints where the patella was dislocated. The surgical procedure in this joint was identical to that used in the joint illustrated in Fig. 22. $\times 2.5$.



19



18



20

E. Piotti

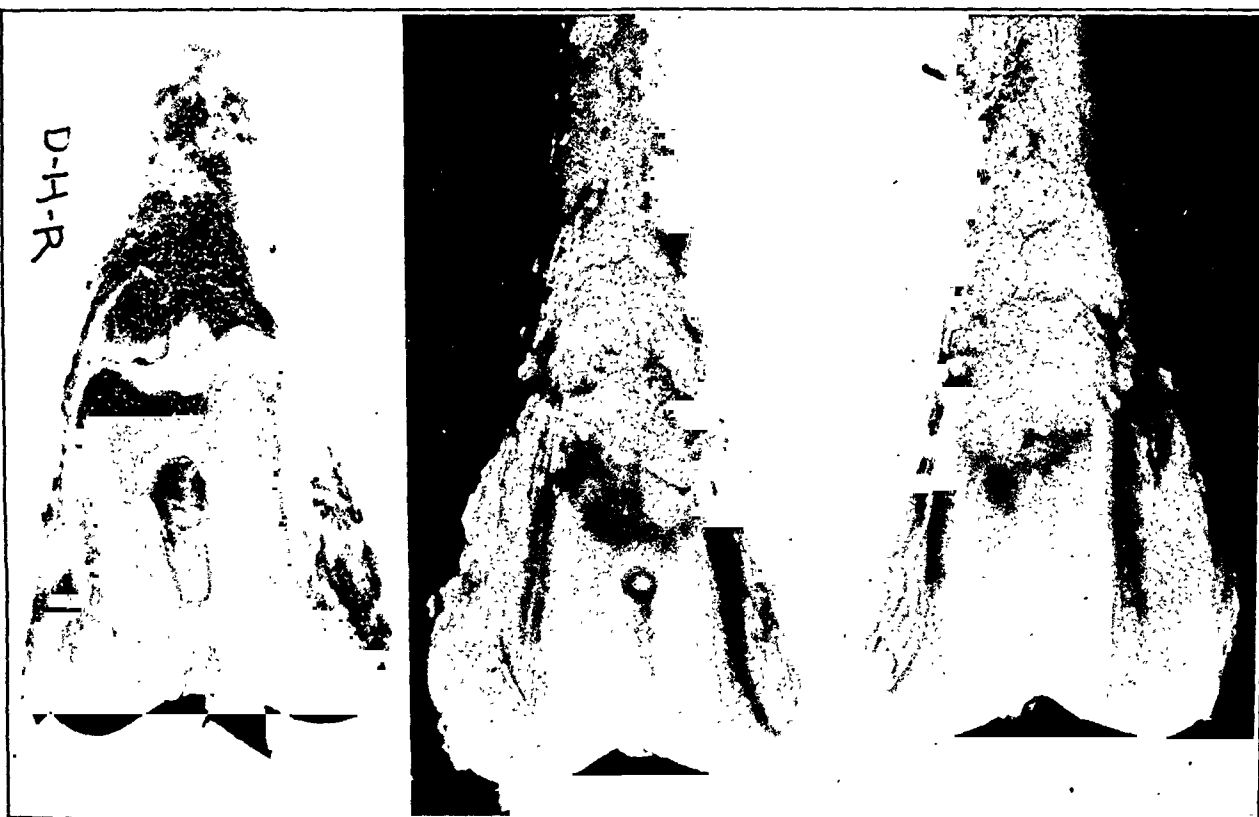


21

PLATE 98

FIG. 25. A low power photomicrograph of a defect in cartilage and subchondral bone after a period of four weeks. Note the absence of proliferation of bone or cartilage. The crater of the defect is filled with fibrous tissue. $\times 76.5$.

FIG. 26. The repair of a defect in cartilage and subchondral bone after a period of twenty weeks is illustrated in this photomicrograph. The fibrous tissue which was found in earlier specimens (Fig. 25) now resembles fibrocartilage and imperfectly formed hyaline cartilage. The matrix of the recently formed tissue and the original cartilage is fused and new bone has largely filled the defect crater.



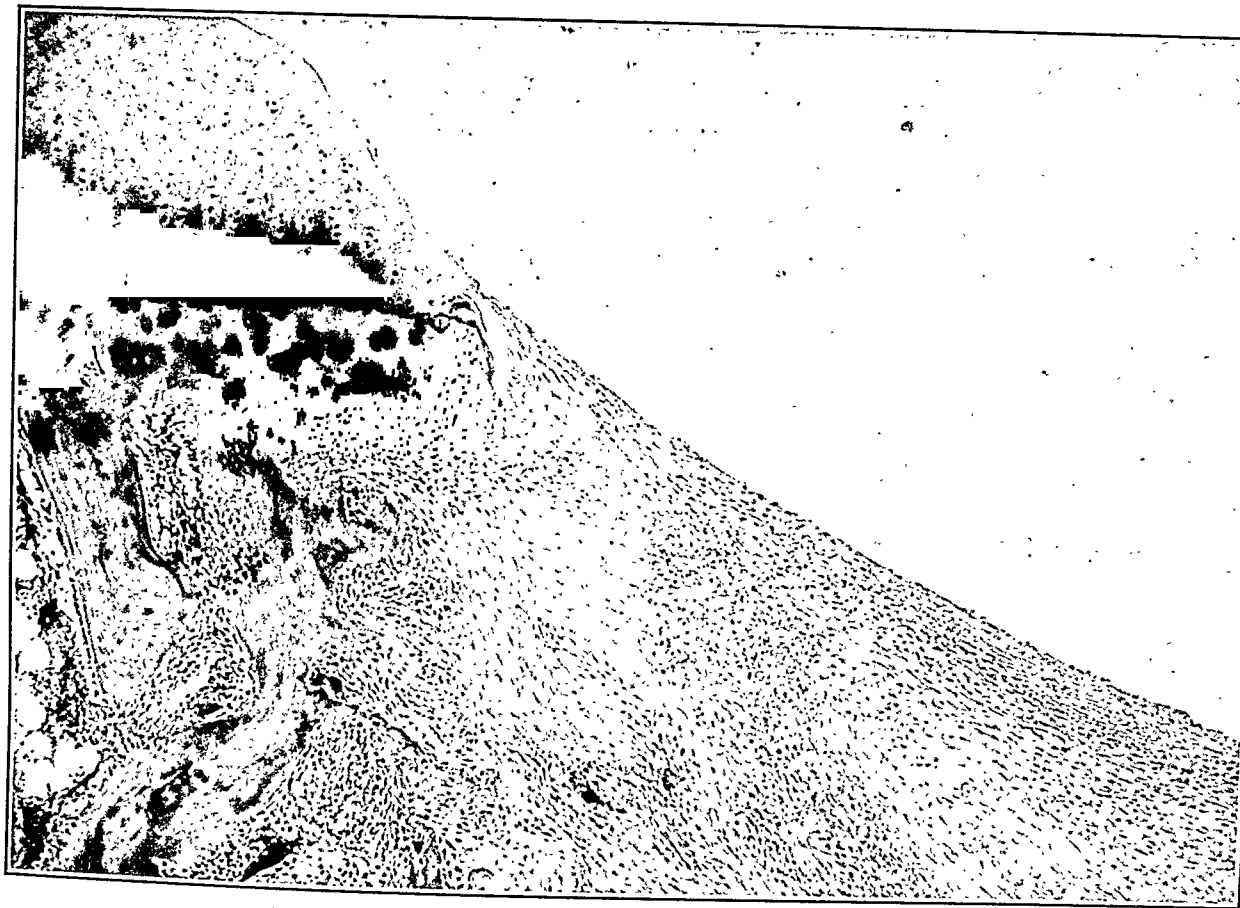
22

23a

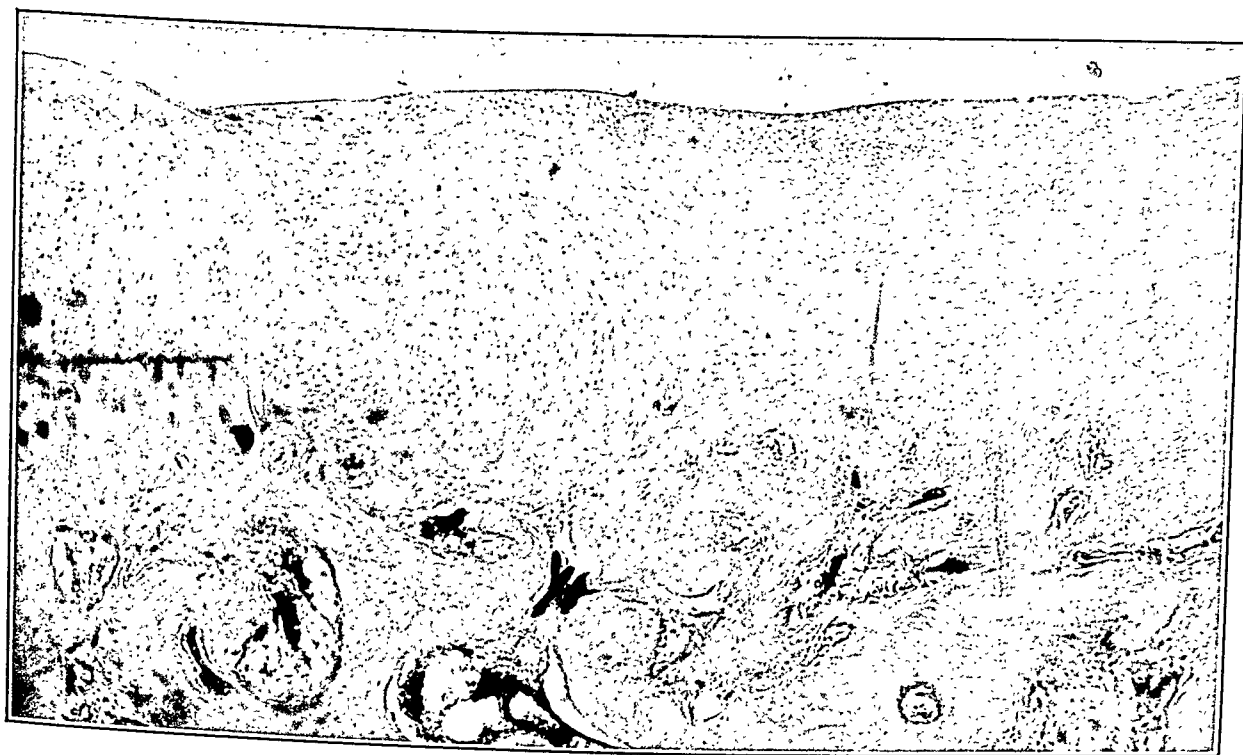
23b



24



25



26

upon the whole dorsal midline a certain lability or even a vulnerability, which paves the way for all sorts of defects and variations. The nominal risks incurred in these extensive changes must be further increased by the peculiar qualities and capacities of the cell layers primarily involved — the central nervous system and the epithelial covering of the body. Not only this, but it may very well be that in human development the dangers are even more enhanced, since the necessary developmental procedures must be carried out on tissues whose natural sensitivity has been further increased by specifically human capacities and susceptibilities. Although this applies primarily to the central nervous system, it is not without its effects elsewhere in the midline.

We are concerned at present, however, not with the gross and extensive defects which are so often found on some part of the dorsum, but with the milder, much less serious cases, and with these again in their incipient stages, as they appear in the human embryo relatively early in its development. There can be no doubt that many, if not all of these cases, from the severest to the mildest, belong in the same category, as visible expressions of some derangement in the normal and orderly growth and differentiation of the various structures and tissues concerned. The severest cases are obviously those in which the neural tube is wide open and as a result many other structures are or would have been markedly deficient. It is not so easy to say, on the contrary, what would characterize the mildest cases; or at what point the normal might be said to grade into the abnormal. This difficulty is especially apparent later in development and in the adult, where the multiplicity of the structures involved, their complexity and natural variability, render difficult, if not quite impossible, any very accurate grading as to the severity of the damage done, or as to the extent of deviation from what might be considered essentially normal.

As far as the specimens under consideration at present are concerned, one might have anticipated rather less difficulty in recognizing a varying degree or extent of injury to the embryo, but conditions are by no means so evident or uncomplicated. Leaving the nervous system out of account, the structure of the body wall dorsal to it is still, in these cases, exceedingly simple and the opportunities for deviations from the normal would appear to be rather limited. But even here, however, during the first two months of develop-

STUDIES IN THE PATHOLOGY OF DEVELOPMENT*

II. SOME ASPECTS OF DEFECTIVE DEVELOPMENT IN THE DORSAL MIDLINE

N. WILLIAM INGALLS, M.D.

(From the Anatomical Laboratory, Western Reserve University, Cleveland, Ohio)

INTRODUCTION

Developmental disorders of various kinds are particularly frequent in the midline of the back. In speaking of the dorsal midline we do not restrict the term to a narrow strip in the axis of the body, but have in mind rather a more extensive area, with variable or even indefinite lateral limits, symmetrically located in respect to the sagittal plane, and extending the entire length of the body, through the head and onto the face. As regards the depth of this region, *i. e.*, its dorso-ventral extent, it is possible to be rather more precise. It may be considered as extending from the nervous system outward to the covering integument, including both of these as well as all of the varied structures which have their proper place somewhere in between. The deviations from the normal or usual conditions, which may be encountered in this region, range all the way from the most inconspicuous variations and anomalies to wholesale defects of great extent. Hardly noticeable and of no importance at one end of the series, they appear at the other end as gross and widespread malformations, whose presence makes independent existence quite out of the question.

The peculiarities of the dorsal midline, its great inherent sensitivity and the special developmental risks which are attendant upon its proper formation, have been considered more in detail in a recent article,¹ but a few of the more important points may be noted here again. As a result of the very radical structural alterations, both gross and histological, each with its genetic implications, and of the relatively massive and far-reaching rearrangements of tissues which occur during the formation of the vertebrate nervous system and the restoration of proper continuity over it, there has been impressed

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in dorsally, a very definite developmental risk has been incurred, and that the parts concerned have had impressed upon them a greater degree of sensitivity and vulnerability than is to be found elsewhere. In the great majority of cases development proceeds normally throughout the body, but disturbed or altered environmental conditions may be expected to compromise or upset, more or less seriously, this orderly development, exactly at those points where the cells and tissues are, for some reason, the most sensitive. There are, early in the life history, particularly in man, two especially vulnerable regions of the body: one is the dorsal midline, the other is the distal portion of the extremities. In view of the normal and inherent susceptibility of these parts to unfavorable environmental influences, it is not necessary to predicate any special germinal, genetic defect to account for most of these cases of maldevelopment. It would seem very probable, however, that this same susceptibility might be a favorable or vulnerable point of attack for various influences which would later appear as germinal or hereditary defects. In any case, the ultimate factors involved are essentially germinal, whether they merely determine an unusual, even dangerous sensitivity, or whether they actually allow or lead to gross malformations. If the genetic composition of the future individual prescribes and carries through a certain normal course of development, then it also and just as certainly provides many of the pitfalls along the way.

The individual specimens to be described below have been arranged roughly in order of the degree of disturbance in the dorsal midline, with some regard also for the general condition and quality of the embryo, beginning with those which show the most pronounced defects and terminating with those where the damage done is relatively slight and perhaps of no very great consequence. The purpose of this arrangement is to call attention to, and to contrast with each other, the major and minor defects which occur in this region. On the one hand the developmental disturbance is at a maximum, with extensive involvement of the nervous system; while on the other it is at a minimum, and only slight alterations in the superficial epithelium can be made out. Between these extremes the order is less trustworthy and of less significance, as already indicated.

ment there is not a little variety in the degree and character of the tissue changes in the midline. These vary considerably in location, extent and apparent severity, as well as in the histological pictures presented. For these reasons it is not often possible to distinguish between earlier or later stages of the same process, if such there be; nor can one always be sure that the real damage to the tissues is greater or more serious in one case than in another. It is altogether probable that the slightest degrees of disturbed development do not appear in this series at all. They would be expected in apparently normal embryos, but only further development would reveal the presence and extent of such latent defects. As long as growth and differentiation might continue there would be the opportunity for these weak or submerged influences to manifest their own peculiar character. In the present series, however, only those cases have been included which show definite and undoubted alterations in the embryo, readily discernible on gross examination.

On account of the importance and significance of the central nervous system in the formation and inherent integrity of the dorsum, and on account of the simplicity of the dorsal structures in the earlier stages, it is convenient to classify the various malformations in this region in terms of the condition of the nervous system. In most of the cases to be considered here, and in all of the more typical ones, the nervous system has been properly formed and closed in behind, while the defects which occur involve only the dorsal body wall, connective tissue and covering epithelium. Since these disturbances in development are much less marked and since they appear only after the neural tube has closed, they may be looked upon as relatively mild derangements. It is not implied, however, that the formation and closure of the neural tube guarantees in any way its own subsequent normal development, any more than that the same would hold for the overlying structures. In these typical, milder cases, the nervous system is to all appearances normal and intact, but the overlying parts give unmistakable evidence of being the site of abnormal processes. What continued development would have brought about may be very problematical, but the visible evidences of the damage already done are confined to the parts dorsal to the nervous system, or even to the superficial epithelium.

One cannot escape the conclusion that in the formation of the central nervous system and of the various structures which close it

specimen, the changes in its walls are more marked than in any other part of the embryo. The form has been preserved but the substance is almost lacking. There has been an extensive cytolysis and the remaining tissue is more like an attenuated mesenchyme than nervous or epithelial tissue. The free, exposed surface is often irregular and indefinite, while the deeper surface seems to merge in many places into the loose underlying mesenchyme.

The vesicle which contained this embryo was rather large, 29 by 27 by 22 mm., the villi were well developed and numerous, but many of them showed early cystic changes. Within the sac there was a large amount of dense, stringy magma which quite obscured the embryo. The amnion was of normal size but much thickened, and presented a large rent through which the embryo had escaped. Except for a few fine tortuous vessels faintly visible on the yolk-sac, there were no vascular channels to be seen anywhere.

Embryo No. 46: (Fig. 2). There was a cessation of menstruation in this case two and a half months before the abortion, but the embryo is much younger than this, for its maximum length is only 14.5 mm. It is markedly stunted and malformed, of a brownish, muddy color, more opaque than normal and has obviously been dead for some time. The body is short and straight, rather cylindrical and gives the impression of being unduly distended. The head is extremely defective, most of the brain seems to be absent, so that the face sets on the anterior part of the trunk with the upper margin of the mouth as the most prominent point. The limbs are fairly well developed although the posterior ones are somewhat retarded. The cord is short and much constricted at its embryonic and chorionic attachments. Between these points it is very greatly dilated, with no evidence of vessels within.

Occupying most of the dorsum of the body and extending forward to within 1 mm. of the eyes is a large pyriform area over which the superficial ectoderm is either lacking or markedly altered. This area is quite symmetrical and measures 9 mm. in length by 6 mm. in width. It is widest in front where it is also raised a little above the general body surface, its limits are quite sharp and even, and it is distinguished from the rest of the embryo by a slight yellowish tinge. In its anterior part this surface is dull and uneven, marked by small, shallow, irregular furrows and depressions or even small cavities. The picture presented is that of a disintegrating surface

DESCRIPTION OF SPECIMENS

The first few specimens which will be considered have been included here simply as illustrations of typical, major midline defects in the embryo. They stand at one end of the series, which at the other fades out into normal or quasinormal conditions. The series presented here might have been extended, a greater variety of cases might have been introduced and the descriptions and histories could have been more detailed, but we have endeavored to cover the more important points in a reasonable amount of space.

Embryo No. 83: (Fig. 1). No history was available. The embryo is rather pale and chalky in appearance and there is a deep tear ventrally between head and trunk, which exposes the heart. Before being damaged the greatest length would have been approximately 7 mm. The head is rather small and there is little relief or detail to be made out. The cord is small and shows no trace of vessels.

Beginning about the first sacral segment and extending almost to the end of tail, there is a wide-open defect in the neural tube. This defect is perfectly regular in outline, measures 2.5 mm. in length and occupies the prominent convexity of the sacral curve extending over eight to ten segments. It is widest near its anterior limit, a little less than 1 mm., while at the opposite end it is a trifle more than 0.5 mm. in width. There is a distinct, sharp linear groove in the center, which does not quite reach the anterior limit of the defect, due to the rolling in here to form the neural tube. At the caudal end the median groove appears to run out onto the dorsum of the tail, and there is no evidence of any attempt at closure or even of the presence of nervous system beyond this point. The widely everted walls of the neural tube, on either side of the midline, are smooth and convex, no details being visible. Their lateral margins are sharp and even and overhang slightly the adjacent body wall.

Sections of the embryo show that extensive disintegrative changes are well underway. These are most noticeable in the head where the brain has suffered most. In some parts of the nervous system, more especially in the cord, the ventral portions seem rather better preserved and less affected than the dorsal parts, but this might be an indication of better nutritive or circulatory conditions in the former. In spite of the fact that the open neural tube in the sacral region was sharply defined and seemed very well preserved in the gross

Embryo No. 129: (Fig. 3). The embryo is represented by a fairly regular, cylindrical mass with rounded ends, 12 mm. in length by 6 mm. in diameter. It is the most stunted and deformed of any of the embryos in this series and shows in addition some of the most interesting surface changes. The color in general is a grayish brown with some darker blotches, while the cord, which comes off the ventral surface at the posterior end of the body, is much lighter. The face, and what remains of the head, make up the anterior hemispherical end of the embryo, the only relief here being found by the wide-open, quadrangular mouth which is completely filled by the tongue. The eyes on both sides show a well defined ring of retinal pigment, with the choroidal fissure pointing toward the mouth.

On the dorsum of the embryo, but much nearer the anterior end, there is a roughly circular, well defined area, which measures about 4.5 mm. in diameter. In spite of the brownish cast and general discoloration of the embryo, this patch, on what should be the vertex or back part of the head, stands out very conspicuously. It is quite symmetrical but a trifle more extensive on the left side along its posterior border. Within this area a little differentiation can be made out, in that the central portion is smoother and rather yellowish in color, while outside of this is a darker, uneven, ragged zone covered over with numerous small appendages or tags of tissue. There is nothing to indicate an open neural tube in this region, the appearance being rather indicative of profound alterations in the superficial tissues.

The sections show that the embryo is much older and more advanced than its gross characters might indicate. The brain and head in general are very defective. The cartilaginous skeleton of the vertebral column and of the larger bones of the extremities is very well defined, but there is a very marked kyphosis, the anterior end of the spine being at right angles to the more posterior portions. The superficial tissues of this embryo, especially in the dorsal area noted above, present a most bizarre and unusual appearance. No attempt will be made at this time to describe in detail these conditions which appear quite foreign and out of place in an embryo of this stage of development. While much of the superficial ectoderm, and in varying extent the underlying tissue also, are very radically altered, the greatest degree of histological distortion and maldevelopment is found in the circumscribed area on the back. There is

tissue, the end results of which are seen in the posterior part of the area. In this later location there is a deep, rather clean defect which was originally filled with prominent, but very irregular masses of degenerating nervous tissue. This tissue was so friable and loosely attached that it was lost in the removal of the embryo from the sac.

Except for the nervous system the body tissues and organs are in better condition than was anticipated. The anterior end of the nervous system shows the characteristic complicated foldings and widespread histolysis and both the pigment and nervous layers of the retina show similar changes. Farther back, as shown in the gross specimen, practically all of the brain and cord has been lost. A study of the sections reveals the fact that the superficial ectoderm is still present over much of the anterior part of the dorsal area which appeared defective upon gross examination. The superficial layers are, as a rule, somewhat thickened, but there are various places where they are quite thin or even entirely wanting. Structurally there is little to be made out definitely, but this region stands in marked contrast with the smooth, even development of the surface epithelium in other parts of the body. It is obvious that the proper development of the ectoderm of the dorsum of the embryo has been radically altered. The original disturbance, whatever it may have been, has not prevented the closure of the neural tube, at least in its anterior part. It has, nevertheless, left its impress on the superficial layers which make up the skin in this region, as shown by its abnormal appearance, both in the gross and microscopic. This compromising of the proper development of the superficial structures in the midline of the back, with little or no involvement of the central nervous system, will be brought out more in detail in the later cases. It is the expression of a greater sensitivity or vulnerability in this region, due primarily to the massive and radical developmental processes which form the central nervous system and the body wall behind it.

The chorionic vesicle is very large, 60 by 40 mm., thin-walled, and shows extensive hemorrhagic areas; most of its surface is covered by thin, adherent decidua and it is filled with a turbid, blood-tinged fluid. The amnion is everywhere in contact with the chorion, but no vessels are to be seen, even in the immediate neighborhood of the embryo. Villi are few in number, small and fibrous.

Embryo No. 665: This specimen is from a first pregnancy at the age of 17 years. There is a history of pernicious vomiting. The menstrual history is somewhat uncertain, but the last period was at least two months before abortion. On account of the damage to the posterior end of the body, the original length cannot be determined, but it was probably not far from 15 mm. The head is small and the details of the face much obscured. Over the anterior end of the head and the upper part of the surface ectoderm is lacking. This defective area is rather extensive and quite symmetrical; it is bounded by a line which runs from about the angle of the mouth upward and backward across the eyes, reaching the midline behind, not far from or just beyond the midbrain. The surface of this area is rather more brownish in color and along its margins the adjacent body epithelium appears loose and slightly elevated. In the midline behind the large superficial defect, in what seems to be the region of the lower rhombencephalon, there is a small, elongated, deep-seated, apparently hemorrhagic spot about 0.5 mm. in length.

Although the ventral thoracic and abdominal walls are torn away, exposing the heart, there are some evidences that the anterior body wall might have been defective over the upper part of the heart, an incipient ectopia cordis: there is also a brownish discoloration here similar to that noted in the head region.

Histological examination reveals a beginning dissociation of the tissues, most noticeable in the nervous system, but the staining reactions are still fairly well preserved. The large defect is seen to be quite devoid of epithelial covering, but along its margins the body ectoderm stops very abruptly and is often heaped up into large prominent cell masses. The cells which make up these conspicuous masses are large and pale, irregularly polyhedral in shape, often several layers deep and seen to be derivatives of, or correspond with, the superficial periderm. There are indications of a similar, but less conspicuous, heaping up of cells along the border of the ventral tear, or the defect over the heart. The surface of the large defect on the head is smooth and even, and the limiting connective tissue cells often appear as a thin, well defined layer of squamous cells. Scattered among the superficial connective tissue cells there are considerable numbers of fairly large, roughly rounded, epithelioid looking cells, with small dark nuclei and of a peculiar brownish pigment,

here an extensive but very irregular thickening of the surface layers, and also what may be called provisionally a widespread hyperkeratosis, with a varying amount of desquamation and the formation of structures which may be characterized as epithelial pearls. The connective tissue beneath is often thick and dense and there are large spaces or clefts, but their relation to epithelium or connective tissue is not always clear. In a few scattered areas there are very definite pigment cells, usually occurring in small clumps. One of the most remarkable features in this specimen is the contrast presented in different tissues in regard to their preservation and in the evidences of continued cell life and activity. Although practically all of the embryo shows advanced histolysis and general disintegration, with the exception of the cartilaginous elements, there are certain regions on the dorsum where the subepithelial structures appear to be made up of perfectly normal healthy cells. It is here that one encounters a rather dense connective tissue, with groups of cells of doubtful significance which often contain conspicuous masses of pigment. The staining quality of the tissues in these regions is quite satisfactory, while everywhere else it is very poor if not entirely wanting. Although the embryo as a whole and almost all of its constituent cells have long since been dead, the cells in the areas just noted seem to have been living practically up to the time of fixation. They form an oasis, as it were, in the desert of death and dissolution around them. They are also peculiar in that they seem to represent a stage in development much in advance of the possible chronological age of the embryo. In this precocity, if the term is permissible, there may be some pathological tendencies, and the same would apply to the hyperkeratosis, so-called, in the overlying ectoderm. The general picture is that of a histological differentiation far in advance of what it should be, a remarkable form of prosoplasia.

The chorionic vesicle is very large, quite out of proportion to the embryo, measuring about 60 by 100 mm. It is invested everywhere by a thick layer of clotted blood. Its internal surface is rough, uneven and dark in color, due to the extensive clots without. The amnion and chorion are fused. The villi are not very numerous, they are thick and fibrous and there is considerable leucocytic infiltration in the surrounding blood. No vascular connections between embryo and chorion are visible.

fect on microscopic examination, although in the gross specimen these were often quite definite and conspicuous. In some of these embryos the surface layers stain very intensely with hematoxylin, while the deeper structures may be quite unaffected. In this particular case the staining is especially intense in the general region of the defect and although structural details cannot be made out, there appears to be no very striking difference between the epidermis here and elsewhere on the body. That the surface epithelium of the back, particularly lower down, has been affected more, or in some other way, than the same layer of cells elsewhere, is indicated by the presence here of subepithelial groups of cells, of epithelial pearls and possibly also by the somewhat different staining reactions. In certain localities also there seem to be considerable amounts of pigment in the epithelium, but it is so masked by the stain that its presence is at least doubtful. Over most of the sacral defect the surface epithelium is quite intact, but near the end of the cord there are several places where it is lacking.

Embryo No. 161: (Fig. 4). No history accompanied this specimen. The embryo is in very poor condition, the head being almost completely detached from the body, the greatest length not far from 12 mm. The head appears small, particularly its anterior end, and in the face only the eyes can be distinguished. On the dorsum of the head, about in the region of the anterior part of the rhombencephalon, there is a transversely elongated, somewhat elevated, uneven mass which measures 3.5 mm. from side to side and 2.5 mm. from before backward. There is nothing to be made out on the surface and the color is substantially like that of the rest of the embryo. The outlines of this dorsal patch are quite definite and it is also fairly symmetrical. Sections through the head of the embryo show practically nothing that can be identified as the area seen in the gross in the back of the head. There is extensive dissociation everywhere although the nuclei still stain fairly well. The superficial ectoderm is very thin and for the most part quite inconspicuous.

The chorionic vesicle is of moderate dimensions, 33 by 26 mm., but it is thin and translucent and there are only a very few long, stringy villi. These villi lie close against the vesicle wall and are all directed the same way, as if smoothed out by some slipping or dislocation of the vesicle. The amnion is loosely fused with the chorion and its cavity contains a large amount of light flocculent precipitate.

but the coloring material is very finely and evenly distributed throughout the whole cell and there are no indications of the usual pigment granules. These cells may account, in part at least, for the darker color of this part of the embryo.

Embryo No. 536: Only the embryo was obtained in this case, which represents the sixth pregnancy in a woman 30 years of age. The first pregnancy went to eight months, and this was followed by four miscarriages, from the fifth to the seventh months. The miscarriage in this last pregnancy occurred six or six and a half months after the last period. During the latter half of this pregnancy the patient was in poor condition, bad tonsils and infected teeth are noted in the history, also the possibility of lues. The placenta was said to be 50 mm. in diameter and to be markedly necrotic.

The greatest length of the embryo is 17.5 mm., its color is poor and there is some shrinkage, the superficial layers show numerous fine wrinkles and seem to be desquamating in many places. The head is rather small. In the sacral and lower lumbar region behind, there is a large, fairly regular area, somewhat more brownish in color, over which the superficial ectoderm seems to be missing. This area is very slightly depressed and its posterior margins are rather sharper and more symmetrical than the anterior. It measures about 4 mm. in length by 3 mm. in width.

Sections through the embryo show the usual dissociation and lack of staining qualities. The brain is much more involved than the cord — even its major subdivision can hardly be recognized. The epidermis varies considerably in thickness and structure in different regions, but it is often made up of several much flattened stratified layers which show a marked tendency to separate from each other and also from the connective tissue below. There is widespread desquamation of the more superficial cells, while much of the epidermis gives the impression of being made up of stiff, hard cells, a condition in some ways not unlike those observed in Embryo No. 129, where there seemed to be a hyperkeratosis. In a few places, on the dorsum anterior to the defect, there are small "epithelial pearls" embedded in the epidermis. In addition, there are small scattered groups of cells which lie close to, or in contact with the deep surface of the epidermis, and in a few instances they seem to have been derived from the adjacent ectoderm. As noted in other cases, it is not always possible to identify the exact limits of the de-

two previous pregnancies, the first going the term four years before, while the second terminated in abortion at about two months, possibly due to a fall. The present, or third, pregnancy also resulted in abortion, some eleven months after the preceding one. The last menstrual period began fourteen weeks before the abortion, but there is a history of menstrual irregularity for the two months preceding the last period, and of occasional morning sickness for two months before the abortion.

Judging from its condition the smaller fetus has been dead for some time, its larger companion is in very good condition but it is slightly distorted and has suffered a little from drying. The larger one is four times as long as the smaller, the sitting heights being 130 and 32.5 mm. respectively. The placenta and membranes of the larger one were not received. The smaller of the twins (No. 597 B), is light yellowish brown in color and the most superficial layers of cells are desquamating in shreds and sheets of considerable size. On both hands, the fingers, which are short and apparently fused together, are encased in what appears to be a much thickened epidermis. The feet seem to be normal, but the legs show a marked ventral convexity and the thighs appear rather short. In the mid-line over, or just behind, the vertex of the head there is a very conspicuous, symmetrical bleb which has an anteroposterior extent of 7 mm. It is translucent and the regular outline of the head can be made out beneath it. The deeper structures are not involved. The cord is very much kinked and twisted, it varies considerably in size in different places, and obviously there has been no circulation through it for some time. Arising from the cord, close to its attachment to the fetus, are several large, thin-walled, almost pedunculated blebs or vesicles.

The placenta is quite out of proportion to the fetus, measuring 100 by 70 mm. Its fetal surface is irregular and nodular in appearance, due to the extensive subchorial hemorrhages. The amniotic fluid was turbid and discolored.

Embryo No. 407: (Fig. 7). This is a rather typical stunted embryo in a large vesicle, with a menstrual age of nearly ten weeks. It was the first pregnancy and came from a woman 38 years old. For two days previous to the abortion there had been bleeding and abdominal pain. The embryo measures 7 mm. crown rump. It is very pale and of quite uniform color throughout. The head is very

The distal half of the cord is much smaller than the proximal part; no vessels can be seen anywhere.

Embryo No. 210: (Fig. 5). In this case there had been eleven previous pregnancies, nine births at term and two miscarriages at the end of the first month. The mother was 40 years old and there was a menstrual history of fifty-one days. No cause was given for the abortion. The embryo, whose greatest length is 15 mm., is considerably deformed, the head is small and the face appears to be fused with the ventral surface of the trunk. The posterior end of the body is small and tapering, the extremities are somewhat retarded. The cord is short and straight, much distended and shows a small, pedunculated appendage near the embryo. In the dorsal midline, over the rhombencephalon there is a large thin-walled bleb, 3 mm. in diameter and elevated about 1 mm. above the surrounding surface of the body. The limiting walls of the bleb are rather steep and slightly undercut where they join the superficial ectoderm. No gross defects are to be seen around or beneath the superficial bleb and the surface layers of the body are elsewhere unaltered.

Although the embryo is in poor condition histologically, the dissociation of tissues is more marked in the region of the head and face than it is farther back, the brain having suffered most severely. Throughout the cord the dorsal half is beginning to break up, but the ventral portion is in much better condition. No definite changes of any kind can be seen in sections through the dorsal bleb. Both the superficial ectoderm and the underlying structures appear unchanged, except for their separation and the irregular foldings in the surface layer. The developmental damage in this instance is relatively slight, manifesting itself simply as an accumulation of fluid underneath the ectoderm. There can be no doubt that even milder disturbances may occur here, as well as elsewhere, but they are latent, in a sense, and become conspicuous or recognizable only later in development.

The vesicle belonging to this embryo is substantially normal as regards its size, but its walls are thin, the villi very poorly developed and there is considerable hemorrhage under the decidual layer, by which it is completely surrounded. No vessels can be seen either in the sac or in the embryo.

Embryo No. 597 B: (Fig. 6). This specimen, from a woman 37 years of age, is the smaller of two fraternal twins. There had been

forehead. Below and internal to the right eye, there is a large pit-like defect.

In the midline behind, in the lower dorsal and lumbar regions, there is a slightly elongated area, about 7 mm. in length, over which the superficial layers seem to be wanting. This area is, in general, quite symmetrical, but a trifle more extensive on the right side. The right border is especially conspicuous, appearing as an irregular, ragged, elevated line of thickened or partially detached epithelium. In the anterior part of this area there is a smaller, darker patch, quite sharply marked off and situated almost exactly in the median line. On the anterior surface of the head, a short distance above the eyes, there is a large and very conspicuous, brownish discolored band, extending almost the entire width of the head. This frontal patch is much more striking in appearance than the one on the dorsum; it is also a little darker in color and more abruptly set off from its surroundings.

The cord is small, short and straight, its distal two-thirds is occupied by a large thin-walled, spindle-shaped enlargement, but at its attachment to both embryo and chorion it is very much constricted. No definite blood-containing vessels can be seen within it.

Sections through the posterior part of the embryo show that over most of the dorsal area noted above, the surface epithelium is intact. It stains very densely so that its structure cannot be determined, but it seems to be rather thicker than elsewhere. Along the margins of the area, however, there are numerous conspicuous thickenings in the ectoderm, as can be seen in Fig. 8. These thickenings are as a rule small and scattered, and they vary much in form and size from broad low mounds of cells, to slender, almost pedunculated outgrowths. The cells appear as if heaped up on the surface, without any involvement of the deeper layers. In general the subepithelial tissue seems rather denser and more fibrous on the back of the embryo, but it does not present the marked alterations to be seen in the frontal region. In the smaller central patch on the back the epithelium is definitely absent, and along its borders the limiting epithelium is thickened and ends abruptly, but the large, exuberant masses which are present farther back and more laterally are not seen here. Not only are the surface cells lacking here, but there is further evidence of disturbed development in the presence of numbers of large, rather wide clefts in the exposed connective tissue.

small, there are no indications of eyes, and only the first branchial arch can be made out. The limb-buds are small but fairly well developed, segmentation is only faintly indicated. Immediately above the anterior extremities there is a large bleb-like swelling on either side. In the dorsal midline, in the lower thoracic region, there is also a conspicuous elevation of the superficial layers over a small circumscribed area. Farther back, opposite the posterior extremities, there is a longer, but much less conspicuous swelling, not noticeable in the illustration.

The tissues of the embryo are in very poor condition, the nervous system, and more particularly its anterior part, being most severely affected. In the region of the superficial changes noted above, the constituent tissues are apparently unaltered, save for the separation of the epithelium from the underlying connective tissue, and the tearing and displacement of the former. All of the structures stain very poorly.

The sac is somewhat distorted and thin-walled. It measures 52 by 35 by 20 mm. It is very pale, practically free from blood and almost completely covered by thin, smooth decidua. Where exposed, the villi are rather slender and scattered but not especially abnormal. The amnion is large and somewhat thickened, and through a rent in it the embryo had escaped into the exocoelom. Blood vessels are not to be seen either in the embryo or in the membranes.

Embryo No. 442: (Fig. 8). The specimen to be described here is a tubal pregnancy from a colored woman 26 years old. Four years ago there had been an abortion at three months, the cause being undetermined. For several years there had been a bilateral salpingitis, also dysuria and frequency of urination for the past three months. The last menstrual period was ten weeks before operation, but throughout most of this time there had been spotting and pain in the left lower quadrant. At operation there was a chronic salpingitis on the right side, the uterus was small and forward and there were simple cysts in both ovaries. No mention is made of a corpus luteum.

Within the tube is a turbid amber fluid, apparently blood-stained. The embryo, which has a greatest length of 26 mm., appears fairly normal, although its color is not good. The eyes are large and conspicuous but the lids appear retarded in their development, due possibly to mechanical interference from the skin defect over the

Although the tissues of the embryo are beginning to dissociate they still stain fairly well. The epithelium over the dorsum of the head is everywhere intact and shows no definite alterations in the arrangement or in the general morphology of its constituent elements. The discolored area seen in the gross specimen is, however, quite recognizable. It appears under low power, stained with eosin, as a fairly well circumscribed, pale yellowish pink region in the superficial ectoderm. There is not the even yellowish tinge seen in some of the connective tissue cells, in Embryo No. 665, or the very evident pigment granules which were present in Embryo No. 129. It looks more as if the region in question had been dusted over with a fine powdery substance, which, although it seems to have no definite relation to the epithelial cells, is confined to them and is not to be seen in the underlying tissues. This material is quite evenly distributed and its limits are rather definite. Scattered through these pinkish areas there are a few small, rather densely staining nuclei, much like those in the deeper tissues, but more numerous here than in the epithelium elsewhere. This peculiar material does not suggest pigment at all; it gives rather the impression of something which had been applied to the cells or even to the sections. The neighboring mesenchyme does not appear to be in any way involved.

The vesicle is essentially normal, the villi are numerous and well developed; the amnion may be slightly thickened and in the exocoelom there is abundant, coarse, stringy magma.

Embryo No. 167: (Fig. 10). Except for a menstrual history of "about ten weeks," and that the abortion was "not induced," there were no data available on this specimen. The embryo is damaged somewhat, especially about the mouth and upper part of the trunk, and its color is decidedly poor. Its greatest length is 18.5 mm. The head, particularly its anterior end, is small, the mouth is wide open and there are deep tears on either side. Between the eyes are a number of small holes, or pit-like defects. The retinal pigment is paler than usual, and appears red rather than black. Both the anterior and posterior extremities, but particularly the former, are much retarded.

In the dorsal midline, about the region of the lower rhombencephalon, there is a small, transversely elongated, somewhat smoother, discolored area, slightly greenish in color. It is roughly

In addition to the spaces in the connective tissue of the dorsum there has been an extensive accumulation of fluid behind and on either side of the posterior end of the spinal cord, so that the latter appears to lie on the ventral wall of the large open cavity. At the very extremity of the cord there is a small irregular diverticulum which comes off from the ventral part of the central canal.

No essential difference can be seen in the epithelium over the frontal discoloration, as compared with that of the dorsal area, but in both cases the details are obscured by the intense staining. The former, however, shows only a very slight thickening of the ectoderm in a few places near the margins of the area. In the underlying tissues, on the contrary, there is a very striking difference. Running through the more superficial part of the sub-epithelial connective tissue there is what appears to be a rather denser stratum of the same tissue, but one which is stained almost as deeply as the ectoderm outside. This stratum is sharply delimited, near the center of the area it lies very close, if not in contact with the epithelium, but elsewhere there is interposed a thin layer of paler, normal connective tissue. Apparently this stratum was responsible for the very obvious and well circumscribed discoloration seen on the forehead in the gross specimen.

Embryo No. 652: (Fig. 9). No menstrual history was obtained for this specimen, which came from a fibroid uterus. Even when fresh the condition of the embryo was not good, the color being a turbid brown, the greatest length a little over 15 mm. There seems to be some desquamation of the surface epithelium, but this may be in part adherent precipitate. The head, especially its anterior part, is small, the mouth is widely open and its lateral angles may be torn somewhat. Trunk and extremities are fairly normal, although the latter appear slightly retarded.

On the dorsum of the head, over the anterior part of the rhombencephalon, there is a rather definite pale orange discoloration. It is symmetrically disposed as a transverse band which is best developed on the right side and somewhat indistinct in the midline. As far as can be seen the surface epithelium is intact and not materially altered.

The cord, which is small and straight, shows a small but prominent bleb close to the embryo. There is little evidence of vessels within the cord.

the epithelium is intact, but there are a few small thickenings around the margins of the area, especially the dorsal area. Over the frontal areas the epithelium appears quite unchanged, but at one or two points there are slight defects, apparently postmortem tears.

The vesicle of No. 167 is of normal size, but it is very thin and villi are practically absent, except for one large clump where they are numerous and thickly set. The villi present are long and somewhat swollen, but there are no globular forms. No vessels can be seen anywhere.

Embryo No. 404: Although this is a tubal pregnancy, the embryo is in much better condition than most of the specimens in this series. Its greatest length is 21.5 mm. and, except as noted below, it appears perfectly normal. This was the third pregnancy in a woman of 27 years. There was nothing out of the ordinary in the first two. The last period was sixty-two days before operation and there had been acute pains in the right lower quadrant for a month. The embryo and amnion were all that were obtained.

The only point to be noted concerns the very posterior end of the body. Here, in the midline of the back, over the lower lumbar and sacral regions, there is an area about 6 mm. long and 2 mm. wide reaching almost to the end of the tail, over which the surface epithelium is apparently missing. The margins of this defect are quite smooth and regular, both its anterior and posterior limits are symmetrically rounded, and there still seems to be some tissue covering the cord. It does not seem possible that this loss of tissue could be due to simple mechanical violence. In the sections there is little to be seen except the absence of the surface cells in the region of the defect. There is some suggestion of a thickening in the epithelium along the margins, but nothing very definite. The exposed connective tissue is unaltered.

Embryo No. 671: (Fig. 11). There are no data on this specimen, except that the abortion was probably self-induced. The embryo, which has a greatest length of 25 mm., is well developed and in fair condition, but there are accumulations of fluid under the epidermis and a number of superficial hemorrhages. Over the posterior aspect of the neck and lower part of the head there is a very extensive area in which the superficial layers are raised up in an enormous bleb. This region is fairly well circumscribed and quite symmetrical. The outlines of the underlying structures, which appear normal, can be

reniform in outline, sharply marked off from the surrounding parts, slightly elevated and measures about 3 by 1 mm. It does not appear to be a simple stain or discoloration and there is no evidence of any injury or defect. Farther forward, over the posterior part of the forebrain, not shown in the illustration, there is a smaller, more yellowish spot, irregular in outline and measuring about 2 mm. in diameter.

A little in advance of this second patch there are still two others of much the same character. These last mentioned, most anterior spots, lie close together, almost in the midline, high up on the forehead. They are rather darker in color, and even more conspicuous than the large area behind.

There is a most striking similarity to be found in the dorsal area of this embryo and the condition noted in Embryo No. 652. In both cases there is a very definite, transversely elongated, more or less discolored area, symmetrically disposed, exactly in the midline and lying over the rhombencephalon. In Embryo No. 167 the area is widest in the median line, while in No. 652 it is narrower there, and rather bilobed in appearance; in the former the area is a little farther back and it is also somewhat more conspicuous. Not only this, but the histological picture seems, with minor exceptions, to be essentially the same in both cases, the chief, and perhaps only, difference being that in No. 167 the histological changes are more marked and also more extensive. There is, in this embryo, the same powdery, or finely granular material which was found in No. 652. It is perhaps a little coarser, it stains more intensely and has more of a purple or violet color. This difference in appearance may be due in part to the larger quantities of the material present and to the fact that all of the tissues are stained more deeply with hematoxylin than in the other embryo. Not only is this material much more abundant and more closely packed, but most of it is found in the connective tissue immediately below the epithelium, whereas in the earlier case it was confined to the epithelium. In the present embryo it is likewise present in the epithelial cells, but it is much more marked in the underlying tissue. In its distribution in this case it seems much more sharply circumscribed, due apparently to the larger and denser masses involved. The three areas over the forebrain show the same features — if anything the involvement of the mesenchyme is even more marked. Here, as in the previous case,

confluence of the spaces thus formed. This is what has evidently happened on the dorsum where there is a very extensive undermining of the surface layers. At the site of the defect, the outer wall of this fluid-filled cavity has given way, or been torn, and in addition some of it is actually missing. This anasarctous condition extends from the posterior part of the head downward along the sides of the neck and behind the shoulders into the lateral body walls. It encroaches only slightly upon the face in front of the ear. The dorsal midline is not involved, except in the region of the head. The connective tissue and covering epithelium show no changes except the stretching and distortion in the former. It should be noted that the condition is most marked over the dorsum, that it is here only that tearing or loss of tissue has occurred, and also that in the other embryos which are affected in this way it is only the back that is involved.

The vesicle measures 30 by 28 mm., not including the villi. It is somewhat shrunk and pale brownish yellow in color. About half of its surface is covered by long, close-set villi, while the remainder is almost bare. The villi are not normal. Many of them are enlarged and swollen, while many show fine threads like branchings. Bulbous and globular enlargements are common, as well as great numbers of fine, short, side branches. The vessels in the larger villi are more conspicuous than usual. The interior of the sac appears normal.

Embryo No. 611: (Fig. 12). This specimen came from a hysterotomy ten weeks after the last period in a woman 31 years old. It was the second pregnancy and was indicated on account of pelvic deformity which had caused considerable difficulty at the time of the first labor. There had been slight nausea for three weeks preceding the operation. The left tube was found markedly adherent to the lateral pelvic walls and there were three cysts attached to its fimbriated end.

The embryo, which measures 23 mm. greatest length, is apparently normal, although its condition is not quite what it might be. There are only a few points which need to be noted. In the midline, over the cerebellum, there is a minute, thin-walled bleb, except for which the entire dorsum is intact and normal. There are some small scattered ecchymoses behind the left ear, on the left shoulder and on the lateral thoracic wall behind. As seen in ventral view there is

made out quite readily through the thin walls of the bleb. On the left side there is a sharply circumscribed mass of blood covering most of the lateral wall of the bleb on the inside. It appears to have simply settled down into this position, due to the embryo lying on the left side. There is widespread hemorrhage in the superficial tissues on the right side of the face and lower part of the head and a few smaller hemorrhages on the left side. Two or three small, deep-seated hemorrhagic spots can be seen within the bleb, which may be the source of the blood clot on the left side. In the lower thoracic and upper lumbar regions there is a separation of the superficial layers over a considerable extent in the midline, but this is much less conspicuous than the conditions just noted and there is no extravasation of blood. Both upper extremities show considerable vascular engorgement and some actual hemorrhage, especially on the right side.

The chorionic vesicle is rather large for the embryo, and is covered practically everywhere with long, thick-set villi. It is not entirely normal, however, since many of the villi are swollen and irregular, and smaller cystic forms are quite plentiful; the vessels within are quite conspicuous.

Embryo No. 513: Like Embryo No. 404 this is also a tubal pregnancy, but it was the first pregnancy in a woman of 32 who had been married for ten years.

Although this embryo is essentially normal it is not in quite so good condition as No. 404. Its greatest length is 18.5 mm. In the midline behind, just below the fourth ventricle, there is a small oval patch about 4 mm. from side to side and 3 mm. in its anteroposterior dimension. It is not exactly in the midline, but slightly to the right, and obviously the surface layers have been torn away.

From the sections one might conclude that the defect on the back of the head is simply a local accident in a process which is much more widespread. This more extensive condition appears as a marked edema in the subepithelial connective tissue. It is peculiar, in that it is quite symmetrical on the two sides of the body and over the dorsum, and also because the great enlargement of the connective tissue spaces is confined to the more central layers of the mesenchyme, the more superficial as well as the deeper layers being unaffected. The later or final stages of the process are represented by the tearing apart of the much attenuated mesenchyme and the

nothing which could be positively identified as the fine line seen at an earlier date. This is the smallest and least noticeable alteration in the midline that we have encountered in any of our cases.

The chorionic vesicle is of fair size, 45 by 26 by 20 mm. Its walls are thin and the villi, which are few in number, are scattered along one side and at one end. Many of the villi are slender and stringy, while others are definitely dilated and bulbous. The amnion is already fused with the chorion.

DISCUSSION

From the foregoing descriptions of seventeen specimens, it is evident that peculiar conditions obtain in the dorsal midline. Early development may be compromised or deranged in a variety of ways, but the effects are most often in evidence in that part of the body from which is formed the central nervous system. Milder derangements may show themselves only at a later date, or only in those structures which cover in the neural tube behind. The extreme susceptibility of the early nervous system to unfavorable influences, whether occurring in nature or under experimental conditions, has long been recognized, while its significance in human development has been abundantly illustrated by Mall,^{2,3} Mall and Meyer,⁴ as well as by many other writers. Much less attention, however, has been given to the comparatively slight defects which abound in this region. From our own experience these milder cases may be more common than the more severe ones, particularly during the first two months of development. The possible significance at the end of development of some of these less conspicuous deviations from the normal will be considered on another occasion, likewise the finer histological details exhibited by the tissues affected.

The extensive works of Mall and Meyer, referred to above, contain numerous examples of defective midline development. They also bring out very clearly the great vulnerability of this region, as shown by the high percentage of cases in which it is mainly or alone involved. While our own series has been selected on the basis of minor defects, and for these only, if they occur in the midline, all sorts and degrees of maldevelopment, regardless of their character or location, are included in Mall's and Meyer's material. For this reason many, perhaps the majority, of their cases of midline defects represent severer degrees of damage than are encountered in our

a fullness about the head, behind and below the ears, suggestive of edema.

From the sections it can be seen that the embryo is not as normal as it appeared to be. In the region of the bleb shown in the illustration, the epithelium is separated from the underlying tissue. Farther forward, however, there is another, even more extensive area in the midline where there is an accumulation of fluid, but in this case it is located deeper, within the connective tissue. In addition to these median spaces there are two wide clefts on either side of the midline over the forebrain. Along the lateral aspects of the brain, farther back, the connective tissue spaces are very much dilated and in many places there are wide-open spaces, the condition being much like that seen in Embryo No. 513, but not as severe. Except for these spaces the tissues appear normal, but most of the blood vessels are very much engorged with blood, even the smallest ones.

The vesicle is rather small and is thickly covered with long, richly branched villi which are often matted together. Most of the villi are swollen and irregularly dilated, but the changes are not as marked as in No. 513.

Embryo No. 682: The present series of cases may be appropriately brought to a close with this specimen. The history is somewhat uncertain, but this appears to have been the first pregnancy. The menstrual age is given as two and a half months and the abortion might have been induced. The color and condition of the embryo are not especially good. The greatest length of the embryo is somewhat uncertain but may be taken as not far from 15 mm. The anterior end of the head seems rather small, the mouth is more widely open than usual. Although blood vessels can be seen in the cord, they are hardly distinguishable at its attachment to the membranes. The cord itself is smaller than normal, and most of it is occupied by a large, irregular bleb. On the dorsal surface of the left foot-plate there is an extensive, diffuse, but rather mild hemorrhage.

Exactly in the midline of the back, in the slight concavity behind the midbrain, just in front of the cerebellum, there is a faint brownish, linear discoloration, at right angles to the median plane and not over 1.5 mm. in length. It appears as a narrow, slightly irregular, pigmented line, but its exact nature cannot be made out. Attempts to photograph this dorsal patch were unsuccessful; not only this, but later examination of the specimen, in the gross, showed little or

they might play in those cases where development is *not* interrupted. It is, of course, not possible to say just what might have been the final result in any particular case. In a few instances one can recognize what may be earlier and later stages of the same condition, but in the individual case there is nothing to indicate whether the process is progressive or regressive or at a standstill. Where the whole embryo is markedly pathological, moribund or even dead, one might suppose that the process would be progressive and terminate only with the death of the cells or tissues involved. In the more normal embryos, however, it would not seem possible to make any prediction, since the greater vitality and growth capacity of the cells might bring about more or less perfect healing on the one hand, or a more pronounced reaction to the causative agent on the other. In some cases the damage seems to be very slight and complete restitution might have been possible, while in others the later stages might have shown local cutaneous defects, or even more deep seated disturbances. Certain aspects of this question will be taken up in a subsequent paper.

In the often sharply located disturbances seen in our specimens there is something akin to the "focal deficiencies" which Streeter⁵ has shown to be of such importance in the development of the extremities. Although the predisposing factors may be different in the two sets of cases, it is quite possible that the more immediate, inciting causes may be more closely related. Certainly the limbs, and more especially their distal segments, show a marked susceptibility to unfavorable influences, and in this respect they are in a class with the midline of the back. The reasons for their exceptional vulnerability, however, are not as apparent.

The recognition of predisposing and exciting factors in maldevelopment is tantamount to saying that normal fertilized ova or normal embryos may give rise to malformations. This is undoubtedly true, and Mall was particularly insistent upon it, but in his writings the emphasis is on the exciting or contributing causes rather than upon the deeper predisposing influences. Streeter is much more specific in his reference to eggs of different quality, of varying capacity or potentiality for development, not only as a whole, but in their various derivatives and at different stages of development. Eggs are no more alike or equal than are the individuals from which they were obtained, or the future forms into

present series. There are, however, in this Carnegie material, a considerable number of cases in which the tissue alterations seem to correspond more or less closely with conditions as we have found them. There is frequent reference to blebs and blisters, loss of superficial epithelium, thickening of the connective tissues, abnormal pigmentation, or the presence of papillomatous outgrowths. Mall speaks repeatedly of ulcerations, particularly on some part of the head, but just how these "ulcers" differ from other defects, which may have a similar location, is not always clear. But neither Mall nor Meyer was especially concerned with any particular type of malformation; the primary object of their investigations was of a more general nature, while the amount and variety of the material to be considered made all but impracticable any detailed account of the findings in each individual case. We cannot be sure, therefore, to what extent the anomalous histological conditions which we have encountered in our material might be duplicated in their specimens. Neither is it always apparent that the defects which they describe are as conspicuous in the gross specimen, as sharply delimited and symmetrical, or as exactly located in the midline as many of those which we have found. Certainly a number of our cases are very similar, even in the gross. Mall³ (Fig. 6b) shows an unusually large and sharply defined bleb on the back of the neck of Embryo No. 1523, while Embryo No. 2261 (Fig. 72) in Plate 5, Mall and Meyer,⁴ is almost a replica of our Embryo No. 671.

Although it is possible to say why the dorsal midline should show a special predisposition to defective development, it is not so easy to say why some of the minor defects should show a predilection for a particular part of the dorsum. It would appear, however, from our own cases as well as from the Carnegie material, that the back of the head, perhaps more exactly the region over the anterior rhombencephalon and midbrain, is more often the seat of defects, usually slight, than any other part of the back of the head. It can hardly be argued that this part is more exposed to external influences than any other. It is more likely that the real explanation is to be found in the inherent factors which govern the growth and differentiation of the human brain and of the tissues which surround it.

We have been interested in these minor defects, partly on account of their relative frequency, but mainly because of the role

sive subchorionic hemorrhages are very common. The obvious circulatory disturbances may be responsible for the presence of hydramnios, early fusion of amion and chorion, changes in the character of the magma and in the composition of the fluid within the vesicle. In addition to the typical midline defects, many of the embryos show other localized anomalies, while in the majority of them the general condition of the embryo has been considerably altered. The internal disorganization and general disruption, which is so often encountered, is in no sense a teratological condition, but rather a pathological one, although the underlying factors may be much the same. Many of the peculiar skin conditions described above are pathological rather than teratological, if one chooses to draw a line between them, for the inherent vulnerability of certain tissues is quite as much a problem for the pathologist as it is for the teratologist. The various anomalous conditions which are exhibited by the superficial tissues are of very great interest. Two points only need be mentioned here. In the first place, the relatively great expanse of surface exposed to the amniotic fluid would seem to provide some measure of sustenance even after the embryonic blood circulation had ceased entirely. This would apply only to the outermost cells of the body, which might, on this account, be able to prolong their life after the death of all of the deeper structures. In other words, the skin or covering cells of the embryo might, under some circumstances, be the last to die off. The other point is that the amniotic fluid might conceivably act as an irritant to the superficial cells. There seems to be evidence that its character and composition may be altered and in some of our cases one gets the impression that the epithelium has not been living and differentiating under normal external conditions. This hypothetical irritation may, of course, be very mild, behaving more like a stimulant than anything else.

Doubtless the fact that the circulation is so often impaired explains why hemorrhages are relatively infrequent. They occur only in the better preserved, more normal specimens, and here mainly in the region of the head. We have found no indications of hemorrhage or bleb formation in the extremities at all comparable with those described by Bagg⁶ in mice. It may very well be that hemorrhage is less frequent on the back than in the hands or feet, on account of the earlier and greater vascularity of the latter. It is also possible

which they might have grown. This perfectly natural and normal variability in eggs or embryos expresses itself, in one form, as a varying susceptibility, or resistance to unfavorable influences, and also in the specific type of reaction which such influences may bring out. In the present series of cases we have been dealing with a natural vulnerability of a certain part of the body. It is altogether probable, however, that this vulnerability is not the same in all cases—it may vary in degree or in location, as well as in the disposition of the tissues to react in one way or another. This variability and vulnerability is essentially germinal in character, and for that reason the hereditary possibilities cannot be overlooked.

As pointed out by Streeter, the quality of the egg, and its inherent germinal integrity, determine very largely whether it will succumb early in life, eke out a more or less precarious and misshapen existence, or continue in health and vigor to old age. Even under the most favorable conditions maldevelopment may occur. The more environmental conditions deviate from the normal, the more severe will be the tax upon those factors which should ensure proper development, and the easier it will be for normally latent influences to make themselves felt.

Although the exact role of environmental disturbances in the etiology of maldevelopment is not always clear, such disturbances are especially frequent and conspicuous in young monsters and pathological embryos. Among others, Mall and Meyer have written extensively on the alterations in the embryonic membranes which are so frequent and characteristic, and which undoubtedly play an important part in disrupting or even terminating normal development. As we have nothing out of the ordinary to contribute here, we shall confine ourselves to a brief survey of some of the more general features presented by our material.

There are many ways in which this material is typical of maldevelopment in the human being. Most of the specimens are from the second month. In the cases where the menstrual age is known, it varies enormously, from five and a half weeks to six months, the average being eleven weeks. As a rule the membranes are not normal; the vesicles are often too large, the villi very frequently show hydatid changes in varying degree, or they may be few and small and fibrous. In many cases there is no evidence of any real vascular connection between the embryo and chorion, while exten-

head, as exhibited in human embryos of the first two months. They are of interest particularly because these cases may very well represent the earliest stages of some of the anomalous conditions which are encountered in this region at term or even at any time of life. In many of our cases the dorsal defects are very slight and could have contributed little or nothing toward the interruption of the pregnancy, which, but for *other* reasons, might have continued to term. What would have been the final outcome in these cases is, of course, very problematical. Apparently there is, in most instances, either early death of the embryo or fetus from other causes, or a complete healing and restitution of the parts affected. It is quite possible, however, that the damage may be so slight that it is entirely overlooked or its real significance may not be recognized. As noted previously, anomalous or defective conditions of the dorsal midline, varying greatly in degree and character, but not sufficiently severe to compromise further growth and development, are by no means uncommon during the early weeks of intra-uterine life. While many, perhaps most of these cases, fail to go to term, and among those which do reach maturity there may be, in some instances, a more or less complete or adequate *restitutio ad integram*, there remain a certain number in which the initial damage has been sufficiently severe, or of such a character as to render repair or suitable compensation difficult if not impossible. These are the cases which assume a definite clinical, often surgical importance. They are characterized not by any uniformity in the pathology, or in the structural features exhibited by the conditions in question, but rather by their predilection for the dorsal midline, perhaps more particularly the region of the head and neck.

that the relatively poor blood supply of the structures dorsal to the nervous system, especially early, may stand in some relation, or contribute something to the frequency of defects in this region.

It may be objected that some of the conditions described in our embryos are essentially postmortem changes and that it is not possible to attach any particular significance to them. There may be some small justification for this criticism, since, in the very nature of the case, we are dealing, for the most part, with material which is neither wholly normal nor perfectly healthy. In many of our cases, indeed in the most interesting and suggestive ones, there can hardly be any question of postmortem alterations and the picture is anything but that of cell death and a cessation of activity. In some instances there is evidence of hyperactivity, rather than anything else, and indeed some dorsal patches described seem to have retained their vitality longer than any other parts of the embryo. Although we have excluded, as far as possible, all cases which seemed to show only maceration or other moribund changes, it is quite possible that these influences may have contributed something to the general character of the picture in some instances. The variety of conditions observed, and the fact that they occur typically and almost exclusively in the dorsal midline, would indicate the importance of internal rather than external factors in their production, and least of all of postmortem influences.

In the first number of this series on the pathology of development (Ingalls,¹) we have considered in some detail the general underlying biological and genetic principles out of which flow, naturally and inevitably, certain developmental risks. These risks, or the opportunities for various developmental derangements, are especially in evidence in the structures dorsal to, and including, the central nervous system. It would appear also that these parts of the body are especially sensitive and susceptible in *man*, and this instability, in a sense this relative vulnerability, may express itself in an almost endless variety of ways. The factors at work here are internal, inherent in the nature of the organism, essentially hereditary in character, although altered or disturbed external, environmental conditions may be necessary for, or at least conducive to, abnormal results.

In the present communication we have been concerned especially with some of the milder types of maldevelopment of the back and

DESCRIPTION OF PLATES

PLATE 99

- FIG. 1. Embryo No. 83. Greatest length about 7 mm. Open neural tube in sacral region. Facial features distorted, heart exposed.
- FIG. 2. Embryo No. 46. Greatest length 14.5 mm. The entire body is very much malformed. Almost the whole of the dorsum is markedly altered or defective. The deep triangular cavity is due to the postmortem loss of tissue.
- FIG. 3. Embryo No. 129. Greatest length 12 mm., dorsal view. Irregular, roughly circular discolored area in the anterior part of the back. Entire body badly stunted and deformed.
- FIG. 4. Embryo No. 161. Greatest length about 12 mm. Symmetrically located, transversely elongated area over the anterior part of the rhombencephalon. Embryo in very poor condition.

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PLATE 100

FIG. 5. Embryo No. 210. Greatest length 15 mm. Large thin-walled bleb in the midline over the rhombencephalon. Much deformity in the body and tail.

FIG. 6. Embryo No. 597 B. The smaller of a pair of binoval twins, greatest length 32.5 mm. Very large, rather thick-walled bleb in the midline of the back just behind vertex. Extensive desquamation, malformed hands and feet.

FIG. 7. Embryo No. 407. Greatest length 7 mm. Small bleb-like elevation of epithelium in the midline of the back. Head small and malformed.

FIG. 8. Embryo No. 442. Greatest length 26 mm. Dark patch in lower dorsal region; on the right and below can be seen the borders of the larger area. Laterally the epidermal thickenings are very conspicuous. There is also a very definite transverse band across the forehead.

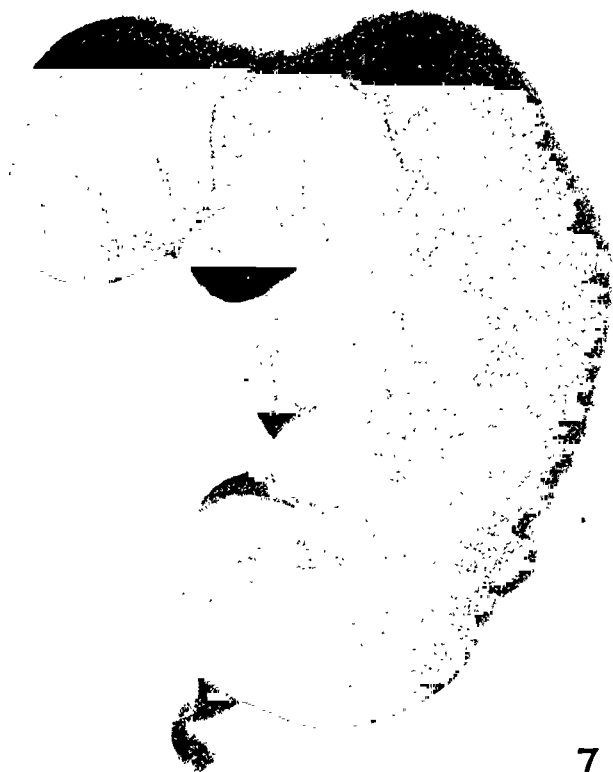


PLATE 101

- FIG. 10. Embryo No. 652. Greatest length 15 mm. Dorsal view of anterior half of embryo to show the transverse discolored band just behind the mid-line.
- FIG. 11. Embryo No. 167. Greatest length 18.5 mm. Very conspicuous, sharply defined, discolored area over the rhombencephalon. There is a similar smaller patch over the vertex and two paired spots on the upper part of the forehead.
- FIG. 11. Embryo No. 671. Greatest length 25 mm. Enormous blood-stained bleb on back of head and neck. In the lower thoracic region there is a much smaller one. Small ecchymoses on face and arm.
- FIG. 12. Embryo No. 611. Greatest length 23 mm. Minute, translucent bleb over the cerebellum. Ecchymoses on head, shoulder and trunk.



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A review of the literature upon this subject indicated to us the opportunity of obtaining much more definite evidence by performing a series of investigations using specialized, improved and more recent techniques such as methods to reveal the mitochondria, Nissl substance and neurofibrils; an improved Millon's reagent which we owe to Prof. R. R. Bensley, the Feulgen reaction for thymonucleic acid as specified by Cowdry¹¹ and the technique of microincineration devised by Policard¹² and improved by Scott.¹³

The Negri bodies were studied (a) in the living state under dark-field illumination, (b) by vital staining, (c) by fixed and stained preparations using selective staining methods and (d) by microincineration. The technique and results of each method will be recorded and the significance of these results will then be discussed.

(a) *In the Living, Unstained State:*

Observations were made on the Negri bodies in the living condition by dissecting out, in physiological saline solution, a small piece of brain tissue from the region of Ammon's horn in a monkey experimentally infected with rabies. The material was then mounted in physiological saline on a clean slide and the coverglass ringed with vaseline. In this manner it was possible to locate large nerve cells containing inclusion bodies.

By ordinary illumination the Negri bodies appear as definite, almost homogeneous masses, in contrast to the very heterogeneous, granular cytoplasm of the cells containing them. The structure of these bodies is ill-defined, except for the presence of a few, rather dense, light yellow granules (chromatoid granules).

Dark-field illumination, using a cardioid condenser, reveals the Negri body as a clear non-refractile body containing granules corresponding in location to the chromatoid granules. No evidence of a vacuolar structure about each granule or of a membrane separating the Negri body from the cytoplasm is visible. Neither are the granules seen to change their location or exhibit movement.

These observations were more in favor of the theory that the inclusions were reactionary products of the cell constituents, rather than that they were protozoan or organismal in nature.

(b) *In Vitally Stained Preparations:*

The application of vital stains, sensitive to oxidation and reduction, to still living nerve cells containing Negri bodies had not previously been attempted, so it was decided to try the effect of

STUDIES ON THE NATURE OF THE NEGRI BODY *

W. P. COVELL, PH.D.,

AND

W. B. C. DANKS, B.S., M.R.C.V.S.

ROCKEFELLER FOUNDATION FELLOW

*(From the Anatomical Laboratories, Washington University School of Medicine,
St. Louis, Mo.)*

In spite of the fact that nearly thirty years have elapsed since Negri¹ described the cytoplasmic inclusions associated with rabies, no agreement has been reached concerning their probable nature. Many investigators have worked on this problem and three distinct views are current at the present time.

1. Negri believed that they were protozoan parasites, a contention repeatedly supported by Williams and Lowden,² and again by Calkins.³ More recently evidence in favor of this view has been brought forward by Levaditi and his associates⁴ who have, on the basis of their interpretation of the morphological and biological properties and evolutionary cycle of the Negri bodies, proposed the name "Glugea lyssae" for them. Manouélian and Viala⁵ have also subscribed to the hypothesis that they are organismal in nature and have considered them to be an aggregation of individual parasites.

2. Other investigators, among whom are Acton and Harvey,⁶ Goodpasture,⁷ and Cowdry,⁸ believe that the Negri bodies arise from injury to certain constituents of the nerve cell brought about by the virus. No agreement has yet been reached as to the actual constituents involved. The neurofibrils and mitochondria (Goodpasture) and the nucleolus (Acton and Harvey) have each been cited as participating in their formation. It has been suggested by Cowdry that a more probable origin is from the Nissl substance.

3. A compromise hypothesis proposed by Prowazek,⁹ concerning the Negri bodies and some of the other inclusions in virus diseases, has recently been upheld by Lipschütz¹⁰ and received skeptically by others. According to this hypothesis the inclusions consist of minute elementary corpuscular organisms enclosed in a mantle of substance produced by the cell in response to their presence, that is, they are chlamydozoa, or mantle animals.

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between the Negri body and its cytoplasmic environment is suggested by the reduction of Janus green B by its hyaline matrix which encloses the non-reducing granules.

Janus red B is reduced to a yellow color by the matrix while the granules remain a deep red.

A comparison of the staining reactions with the two Janus dyes and brilliant cresyl blue denotes a less permanent staining on the part of the former dyes with fewer differences in range of color. In other words, the chemical nature of the brilliant cresyl blue permits of a diversity of reaction, the result being that such a dye is of value in locating Negri bodies in brain material from animals suspected of rabic symptoms. The disappearance by fading in moist preparations with the two Janus dyes was essentially similar, the staining of the chromatoid granules being the last to fade.

In addition to yielding information concerning the differential rate of reduction within the Negri body, coloration with Janus green B showed the mitochondria with great distinctness. It is true that the chromatoid granules stained a green of the same intensity as the mitochondria, but after the fading of the mitochondria, the chromatoid granules were found to persist for several hours under proper manipulation.

It is of interest to note the similarity in vital staining between this inclusion and that of vaccinia as described by Cowdry, who came to the conclusion that there was no indication of the presence of independent microorganisms within the latter. The concentration of the Janus dyes used was so high that they stained other elements as well as mitochondria. There was obviously a difference in the reaction to the stain between the mitochondria and the chromatoid granules which did not suggest a common origin as contended by Goodpasture.

(c) *In Fixed and Stained Preparations:*

For the purpose of studying the possible part played by the various nerve cell constituents in the formation of the Negri body, materials, in which either fixed or street viruses were used as the infecting agent, were fixed in the following ways: Regaud's formalin-bichromate and Bensley's acetic-osmic-bichromate mixtures for mitochondria, the Hirschler and da Fano techniques for Golgi apparatus, Bethe's method and Cajal's silver pyridine method for

brilliant cresyl blue, Janus green B and Janus red B upon the fresh inclusion bodies, note the staining reaction of their various component parts and possibly gain some information regarding their probable nature.

For this purpose a 1 per cent solution of the dye in 95 per cent alcohol was filmed over a clean slide and allowed to dry. A small piece of Ammon's horn from a rabid monkey was mounted on the slide and spread by means of a platinum loop. A coverslip was then placed over the preparation and ringed with vaseline. As soon as the Negri bodies were located the degree of staining was examined and further changes, like fading and reduction of the dye, were recorded.

Brilliant Cresyl Blue (N. A. C.): With this stain the Negri body as a whole stains a Bremen blue color,* the "chromatoid granules" a Paris blue and the central mass a soft bluish violet. About each of the denser staining components is a less intensely colored region of hyaline material. This imparts to the Negri body a vacuolar appearance about each chromatoid granule, as well as a similarly stained but larger vacuole about the central mass.

In moist preparations the staining was transient, lasting fifteen to thirty minutes; the Bremen blue background of the Negri body was the first to fade and was followed by fading of the granules and central mass.

Fixed preparations which had been air-dried and rapidly dehydrated in absolute alcohol, cleared in xylol, and mounted in balsam, retained the typical staining of the Negri body for several months. Exposure to a carbon arc or other intense sources of light may cause noticeable fading of the staining in from one-half to one hour.

Janus Green B (Grubler): By the use of this dye, employed in the same way as the previous one, it was thought that something characteristic of the Negri body might be found. This dye is capable of being reduced to a pinkish color and then a leucobase, as described by Cowdry.¹⁴ On examination of material treated in this manner bodies were seen in which green granules, corresponding to the chromatoid granules in location and appearance, were embedded in a faint homogeneous material, the latter being the hyaline-like substance of the Negri body. That there is an exchange of substance

* The colors were determined by comparison with Ridgway's standard color scale. (Color Standards and Nomenclature, Robert Ridgway; Washington, D. C., 1912.)

In view of the fact that a negative reaction for the Negri body had been reported by Paul and Schweinburg¹⁵ and that normal nuclei of Ammon's horn give a relatively weak reaction, control material (spleen and liver) was subjected to the same treatment to assure ourselves that the reagents used were suitable. It was found that variable results were obtained. In some Negri bodies the reaction was negative, while in others a faintly positive result was secured corresponding in location to the chromatoid granules within the inclusion.

It was then decided to apply the Millon test, with reagents prepared under the direction of Dr. R. R. Bensley, to sections of brain tissue of rabbits containing Negri bodies. The results were found to agree closely with those secured by the Feulgen reaction, *i. e.*, there were occasional faintly positive reactions on the part of individual granules within the inclusion bodies. It is of interest to note the instances in which a feeble reaction is obtained, indicating the presence of proteins probably somewhat altered or reduced in amount, due to the degenerative changes occurring in the cell.

Macallum's iron reaction, as modified by Nicholson,¹⁶ was applied to material fixed in 95 per cent alcohol. A very faint reaction on the part of the chromatoid granules and central mass indicated the presence in them of masked iron.

The variability of the Feulgen reaction does not agree with the protozoan hypothesis, as presumably this reaction would have been more consistent were this the case; therefore, we interpreted the result of this test and the Millon test as indicating the presence in some inclusions of chromatin of nuclear origin which was not present in others, for example the "lyssa bodies."

The changes which occurred in the various cell constituents did not aid materially in reaching a decision as to which was mainly involved in the formation of the Negri body, if such a phenomenon occurred. However, the earliest forms of Negri bodies were seen to lie in a cap of Nissl substance, which indicated that possibly this observation had some significance in view of the results of the Feulgen test and the masked iron reaction.

(d) *In Incinerated Sections:*

The inorganic constituents of the Negri body were investigated by the technique of microincineration. It was hoped that by so doing decisive evidence would be secured bearing upon the question

neurofibrils, alcohol fixation for the Nissl substance and Zenker's acetic and formol mixtures for general topographic details.

Preparations stained with aniline acid fuchsin and methyl green showed that the mitochondria, in the case of a monkey infected with street virus, had undergone marked degeneration in some cells. The same was found to be true in cells affected by the fixed virus. Marked changes sometimes occurred in the shape of the mitochondria in the area of the cytoplasm nearest the inclusion body, but possible transition forms between mitochondria and Negri bodies were not observed. Neurofibrils, when examined in preparations treated by the methods mentioned above, were usually noticeably altered, showing fragmentation, thickening and dissolution.

The Golgi apparatus, also, was considerably fragmented in a few of the cells containing inclusions. Examinations of sections prepared for the demonstration of Nissl substance by staining with toluidin blue showed, in cells containing inclusions, a marked variability in the appearance and amount of this constituent, from a state in which most of it appeared to have gone into solution to a condition in which it was very little altered. In the lesions caused by the "fixed" virus of rabies small Negri bodies were frequently seen in a cell at one pole of the nucleus, usually nearest the axone, lying closely adherent to it and embedded in a nuclear cap of Nissl substance.

It was considered essential to determine whether or not the Negri body contains thymonucleic acid, by applying the Feulgen reaction, because considerable emphasis has been laid upon its chromatin content by supporters of the protozoan hypothesis. If a positive reaction were obtained, two alternatives should be considered, each in support of widely divergent opinions; (a) that it is organismal in nature, or (b) derived from nuclear constituents present in the normal nerve cell.

The material used in this test was obtained from rabbits and monkeys reacting to inoculation with street virus. Portions of Ammon's horn were fixed in equal parts of absolute alcohol and saturated aqueous corrosive sublimate. Sections 3 to 5 microns thick were cut and mounted and the Feulgen reaction applied to them, as described by Cowdry.

ing such positions within the cytoplasm as to leave no doubt in our minds that they constituted the residue of incinerated Negri bodies (Figs. 5 and 6).

It was impossible to reproduce in the photographs all the details observed in the sections. It can be seen, however, that there is a decided difference in the ash of the nucleus and cytoplasm of cells containing Negri bodies (Figs. 5 and 6), and those without inclusions or of normal cells (Figs. 3 and 4). Camera lucida drawings were made of incinerated cells containing Negri bodies and similarly treated normal cells which show more clearly the difference in the orientation and amount of ash (Figs. 7 and 8).

In cells containing Negri bodies there appears to be an orientation of the ash around the periphery of the nucleus and the cytoplasmic ash is very much reduced, apart from that of the inclusion. There also appears to be a reduction in the amount of ash in the nucleolus.

In a number of Negri bodies examined there was a variation in the organically bound iron present. Stages from a practically iron-free deposit to others in which the iron particles were scattered throughout the cell body, giving it a distinct yellow appearance, were observed. It is clear, therefore, that the incineration of Negri bodies at high temperatures leaves a grayish white ash consisting mainly of calcium, together with a variable amount of organically bound iron ash.

There was no suggestion of any inorganic residue which might represent a membrane surrounding a protozoan, neither did the internal structure of this fairly compact ash, which resulted from the incineration of the Negri body, suggest an aggregation of individual parasites within the body. This was of interest because Scott and Horning¹⁷ in their work on opalinids found that the nuclei of protozoa contained very little ash; and no almost ash-free structure, representing a possible protozoan nucleus, could be distinguished within the incinerated Negri body. The discovery of yellow "masked" iron within some of the inclusions supports the results obtained with the Feulgen, Millon and Macallum reactions and will be referred to in the discussion.

as to whether it is protozoan in nature, or a product of cellular constituents altered by the action of the virus.

The technique employed was that devised by Policard and modified by Scott. The material used was taken from the region of the hippocampus of a monkey which was experimentally infected with street virus and sacrificed 18 days after inoculation. This material was fixed for 24 hours in a solution containing 9 parts of absolute alcohol to 1 part of formalin, after which it was thoroughly dehydrated in absolute alcohol, cleared in xylol and mounted in paraffin. Sections 4 microns thick were cut and mounted on slides, using liquid petroleum to prevent any absorption of water. The slides were then placed in a quartz oven, the heat of which could be regulated by means of a rheostat, and incinerated for 35 minutes at temperatures gradually increasing from 143° C to 604° C. After cooling they were removed and mounted dry. Alternate sections to those incinerated were stained in the usual manner for controls.

The incinerated sections were examined under high dry and oil immersion lenses, using dark-field illumination. The results obtained by this method proved very satisfactory because examination of the control sections showed that the finest topographic details had not been lost by the incineration. The ashes left from the inorganic and organically bound salts within the nerve cells were clearly demonstrable and the cell membranes, nuclear membranes and nucleoli were very evident (Figs. 1 and 2).

The nature of this ash is to some extent revealed by its color. Calcium, magnesium and aluminium appear as a grayish white deposit, organically bound iron as a faintly yellow ash, while free iron is reddish in color.

The distribution of the ash in sections of normal nerve cells examined showed that the nucleolus left a distinct yellow ash, indicating that iron-containing protein is present in comparatively large amounts in that structure. The nucleus contained mainly a deposit of grayish white ash in which a fair number of yellow particles were apparent; and the cytoplasm a large quantity of grayish white ash, probably mostly calcium, with a much smaller, scarcely visible amount of yellow residue.

Similar areas of a control section showing numerous Negri bodies were compared with those of the incinerated material. In the latter a number of cells were seen to exhibit compact ash deposits occupy-

2. The concept that the Negri bodies are formed from constituents present in the normal nerve cell is more in agreement with our findings. Evidence has already been brought forward that the neurofibrils and mitochondria (Goodpasture), and the nucleolus (Acton and Harvey), take part in their development. It has also been suggested by Cowdry that the Nissl substance may contribute to their formation.

With regard to the contention that the mitochondria and neurofibrils give rise to the formation of this inclusion body, it does not seem to us that the evidence is very conclusive, especially regarding the former. The material we used for microincineration had previously been fixed in an absolute alcohol mixture in which the mitochondria would in all probability have undergone solution, and corresponding ash-free areas, occupying the position of the inner bodies (chromatoid granules), to which they are alleged to give rise, would have been present in the incinerated Negri bodies. These were not present. In the stained control sections from material treated in the same manner these inner bodies were clearly demonstrable, which would further indicate that their origin from mitochondria was highly improbable. Moreover, there was the evidence from vital staining which indicated a difference in the chemical nature of these elements, the chromatoid granules retaining their staining for some time after the mitochondrial staining had faded. The former occasionally reacted positively to Macallum's test for masked iron and the Feulgen reaction, whereas the latter never did, and again, the chromatoid granules are basophilic while the mitochondria are acidophilic. Evidence is therefore lacking of transition between the mitochondria and the chromatoid granules.

Although the neurofibrils showed marked degeneration we could not distinguish within the Negri body, located in a cell showing these changes, any sign of material suggesting the formation of its hyaline substance from this constituent. However, the evidence of Goodpasture is more suggestive regarding the neurofibrils, and the possibility of their playing some part in the formation of the hyaline substance of the Negri body cannot be disregarded. The difficulty is that the neurofibrils are seldom, if ever, visible in the living nerve cells of mammals, while the Negri bodies always are. There is reason to believe, as Cowdry¹⁸ insists, that methods of impregnation like those we have employed tend to exaggerate their size so

DISCUSSION

These observations have, we believe, a definite bearing upon the three hypotheses concerning the nature of the Negri body.

1. The hypothesis advanced by many authors that the Negri bodies are individual protozoa, or by others that they are an aggregation of individual parasites does not, in our opinion, appear to be supported by the results obtained in these experiments.

Under dark-field illumination in the living state no evidence of a membrane separating the Negri body from the cytoplasm of the cell was seen, neither did the granules change their location or exhibit movement. The microincineration technique, by which a plasma membrane is rendered visible when present, also supported the previous observation by being negative in this respect. Furthermore, the nucleus of a protozoan (opalina) has been observed by Scott and Horning to contain very little ash, which did not agree with the rather compact ash residue within the Negri body; but of course it does not follow that all protozoan nuclei are characterized by a light ash, so that this observation, though of considerable interest, is of little value as evidence.

The variability in the Macallum, Feulgen and Millon reactions was less in favor of the protozoan contention than of the origin of the inner bodies from extruded basophilic nuclear chromatin and Nissl substance, unless it is assumed that the Negri bodies are dead and dying protozoa.

Again, the observations of other authors, such as Acton and Harvey, are not in agreement with this theory. They state that the extreme variations in size and appearance of these inclusions in the various experimental animals suggest their formation from cytoplasmic material, rather than the interpretation that they are protozoan in nature. It is true that no protozoan has ever been reported which at times is invisible and which exhibits such profound differences in size and appearance when parasitic in different species of animals. The variation between the "lyssa body," as described by Goodpasture, and the typical Negri body would also suggest the same conclusion. Neither does the experimental evidence, which we do not intend to discuss in this paper, lend strength to the idea that the Negri bodies are protozoan.

that in the cortical motor cells of an animal suffering from rabies there was evidently a conversion of the iron-holding basophilic chromatin into oxyphilic granules containing very little iron, and these oxyphilic granules corresponded to the previous location of the Nissl substance. It is known that the Nissl substance undergoes characteristic changes during fatigue and injury, and Nicholson, in his extremely interesting work upon the changes in iron substance in nerve cells, pointed out that the alteration in the iron-reacting granules of the cytoplasm corresponded with the morphological changes in the Nissl substance in nerve cells following injury. It is also pointed out that a regeneration of iron-holding chromatin occurs apparently from the nucleus and in all probability the Nissl substance is eventually replaced around the nucleus if complete recovery of the cell occurs. We maintain that this evidence is in support of our observations on the origin of the "lyssa bodies" and on the variation in amount of Nissl substance in nerve cells containing either "lyssa bodies" or Negri bodies.

According to Scott,²² apart from the Nissl substance, the covering of the nucleolus and the nuclear chromatin also contains iron. He found that in pig embryos, 7 mm. to 18 mm., the iron-holding chromatin is confined to the nucleus, but that during further development the basophilic iron-holding chromatin passes into the cytoplasm but the oxyphilic does not. Other authors believe that there is a passage of the iron-containing protein of the nucleus through the nuclear membrane into the cytoplasm during chromatolysis; this phenomenon might be expected to occur as an endeavor on the part of the cell to preserve a nucleocytoplasmic ratio compatible with life.

It is very significant that in the incinerated sections of cells containing Negri bodies there is an orientation of the nuclear ash around the periphery of the nucleus, and within the Negri body in the same cell it is easy to detect the yellowish ash, which represents the organically bound iron. Likewise, when present, the chromatoid granules of the Negri body invariably show a basophilic staining reaction similar to the basophilic nuclear chromatin. Again the Feulgen and Millon techniques gave a faintly positive reaction corresponding in location to those basophilic granules within the inclusion.

If we accept the observations of Scott, Nicholson and other au-

that apparent similarities are to be discounted. Obviously to reach a decision the two must be compared side by side in the same cells by a variety of techniques in addition to the one used by Goodpasture which, like the silver method, reveals the neurofibrils in an unusually robust state. So elusive are the neurofibrils that we know nothing of their chemical composition and consequently cannot speak with assurance regarding their change into other substances.

The Golgi apparatus, although fragmented in many cells containing inclusions, did not appear to have any relation to their origin.

However, the results we obtained from the microincineration technique and masked iron reaction indicated the presence of organically bound iron, and the strikingly similar results from the Feulgen and Millon's reactions indicated the presence of chromatin of nuclear origin. Both types of reactions occurred within the typical Negri body in the position of the inner bodies or chromatoid granules, and were interpreted by us to be due to these bodies having their origin in the basophilic, iron-containing protein of the nucleus which had been extruded into the cytoplasm during the cellular reaction to the virus. The Negri bodies which gave a negative reaction to the above techniques we interpreted, on the basis of Macallum's theory on the conversion of basophilic chromatin into oxyphilic granules, as consisting of material which had lost its basophilic properties and become oxyphilic. Inclusion bodies which are acidophilic include the presumably atypical inclusions which Goodpasture terms "lyssa bodies."

The presence of organically bound iron within the typical Negri bodies led us to review the available literature upon this side of the question. A great deal of work has been done in connection with the iron-holding chromatin of the cytoplasm (Nissl substance) and the chromatin of the nucleus. As early as 1897 Mackenzie¹⁹ and Macallum^{20, 21} showed that this so-called chromidial substance was rich in iron, and the former was the first to report the presence of organically bound iron in nerve cells. He further pointed out the relationship between the iron-holding Nissl substance (chromidial substance) and the iron-containing chromatin of the nucleus. In his work upon the nerve cells of rabbits which had been inoculated with rabies he found that as long as basophilic granulations were present in the cells, iron-holding material was also present. He further reported a factor of importance to our interpretation, in so far

do exist one would expect them to be at least slightly basophilic, for the tiniest organisms, the Rickettsia, are basophilic. Yet in many Negri bodies no trace of basophilia can be distinguished. For reasons such as these the chlamydozoal hypothesis can only be dismissed as not proved.

CONCLUSIONS

1. The cytological evidence presented, with that of other authors, together with the experimental evidence, is not compatible with the protozoan or organismal theories concerning the nature of the Negri bodies.

2. The contention that they arise from constituents already present in the nerve cell as a result of the action of the virus is in agreement with our findings, but we consider the evidence for the participation of the mitochondria, neurofibrils and nucleolus as inconclusive.

3. Both the Negri bodies and the smaller atypical lyssa bodies are probably formed by alterations in the basophilic Nissl substance, the fundamental ground substance of the cell, and by addition of variable amounts of basophilic material of nuclear origin. There is no evidence that organisms on the borderline of microscopic visibility are cloaked with these cellular components in accordance with the chlamydozoal hypothesis.

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thors, it seems to us that there is now sufficient evidence to account for the origin of the Negri body with its many variations in structure.

It is our belief that owing to the action of the virus (enzymatic, catalytic or otherwise) there is a reaction bringing about chromatolysis with its accompanying alteration of the Nissl substance and chromidiosis. The basophilic iron-containing protein of the nucleus is extruded into the cytoplasm, probably in an endeavor to support the proper nucleocytoplasmic ratio; this in turn is also gradually converted, during the cellular reaction, into the oxyphilic, apparently homogeneous material of the matrix of the Negri body; the remaining unaltered portion or portions forming the basophilic iron-containing inner bodies.

The "lyssa bodies" may, in our opinion, either result from the complete conversion of all this basophilic chromatin into oxyphilic material, or by an aggregation of the particles of altered Nissl substance which during chromatolysis had lost its iron-containing properties.

The remarkable similarity of the variations in the results obtained with the microincineration, masked iron, Feulgen and Millon techniques appeared to us to bear a striking resemblance to those which would be produced as a result of the formation of the various types of Negri bodies, including the "lyssa bodies," from the basophilic nuclear chromatin and Nissl substance due to the action of the virus of rabies. Obviously, however, it cannot be said that either Negri bodies or lyssa bodies are formed wholly from basophilic material. It is impossible to exclude the ground substance of the cytoplasm, containing the Nissl substance, which does not color with basic dyes, for the material of the Nissl bodies is probably present during life in solution in it (Cowdry²³).

3. The compromise hypothesis that the Negri bodies consist of minute elementary corpuscular organisms enclosed in a mantle of substance produced by the cell in response to their action is, owing to its very nature, difficult of proof or disproof. If the elementary corpuscles were microscopically visible it would not be so difficult. The trouble is that we and others have consistently failed to find them. They are by definition different from the "chromatoid granules" which are seldom very numerous and which vary in size, sometimes being comparatively large. If such elementary organisms

DESCRIPTION OF PLATES

PLATE 102

FIGS. 1 and 2. Showing the comparison between a stained control section and an incinerated section taken from the same portion of brain tissue. The fact that the majority of the histological detail is preserved after incineration can be seen in spite of the loss of detail due to refraction of the light upon the mineral salts during photography.

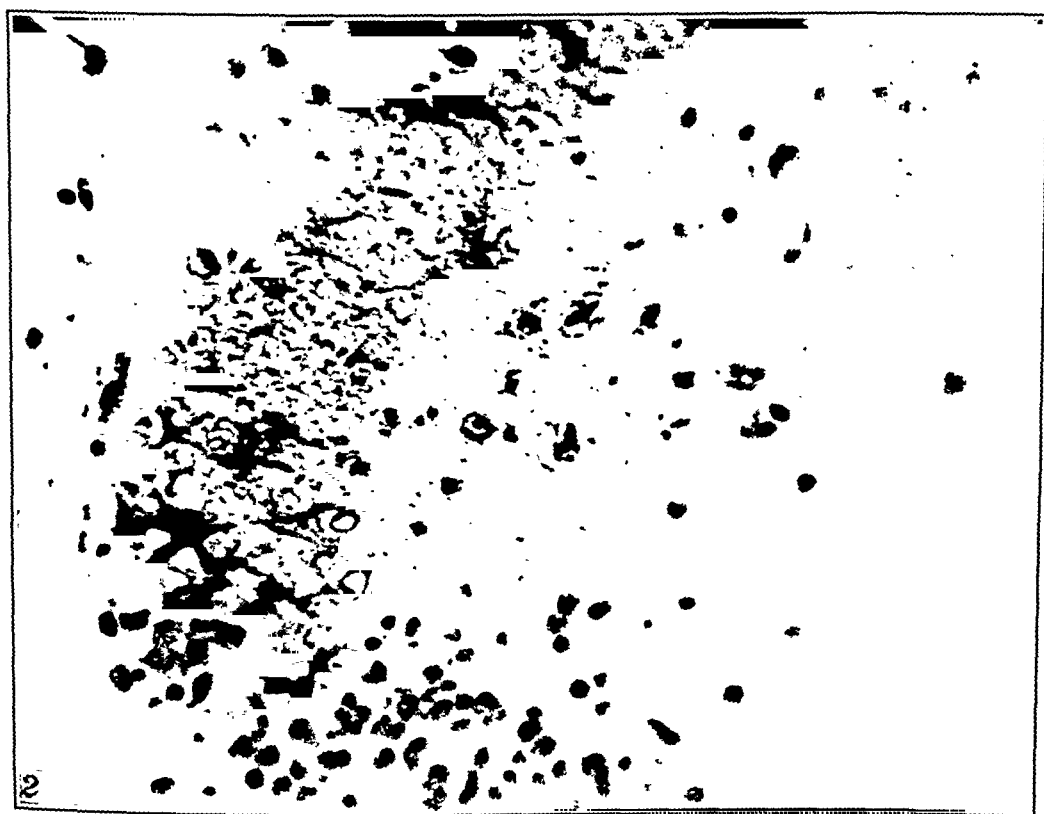
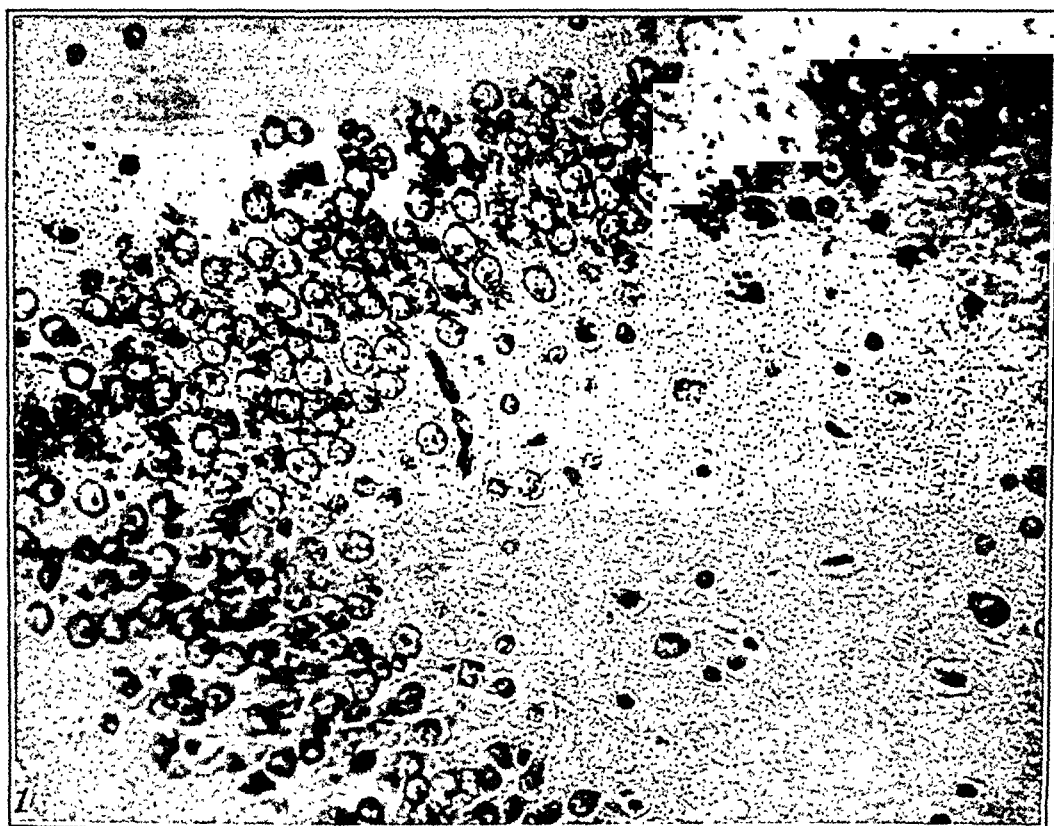
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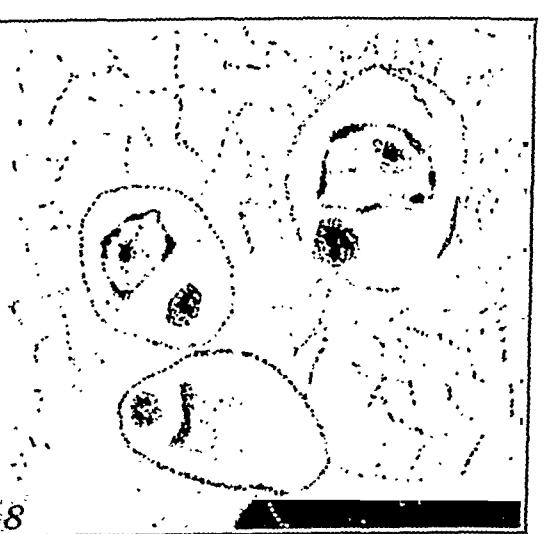
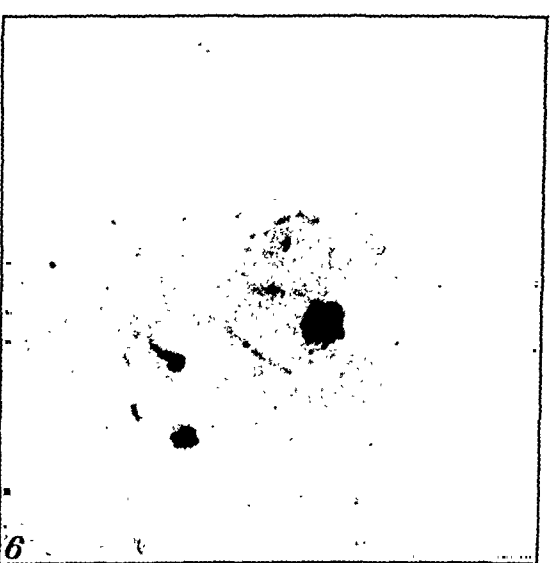
PLATE 103

Fig. 14. Normal incinerated nerve cells showing the amount of mineral present in the nucleus and cytoplasm.

Fig. 15. Cells showing the rather compact ash deposit, lying close to the nucleus, which represents the mineral salt content of the Negri body.

Fig. 16 and 17. Camera lucida drawings indicating the difference in the inorganic ash after incineration of normal nerve cells (7), and cells containing Negri bodies (8). Note the orientation of the ash of the nucleus around its periphery in the latter and also the loss of the cytoplasmic mineral salts, apart from those in the Negri body.





was also emphasized that if an island were large, more than one arteriole supplied its network — as many as three arterioles to some of the larger ones. These short direct arterioles to the islands were contrasted with the longer ones supplying the outlying acinar tissues of the lobule.

It was explained that this arrangement favored the circulation of a large volume of blood through the islands. Since the arterial pressure diminishes directly with the length of the vessel, it is higher at the end of a short arteriole than at the end of a longer one. Therefore, the short direct arterioles to the islands afford a higher pressure for the column of blood entering the capillary network than the longer arterioles supplying the outlying acinar tissues. On the venous side the short direct venules of the islands ensure a low venous pressure with a minimal resistance to the egress of blood. As a result of these observations it could be stated that in chronic passive congestion the circulation through the islands is maintained because the pressure in the short arterioles is sufficient to overcome the increased pressure in the venous system. On the other hand, there is stasis in the peripheral parts of the lobules because the pressure in the longer vessels is not sufficient to overcome the venous pressure.

In the following experiments increased venous pressure in the veins of the pancreas of rats was produced by partially obstructing the flow through the inferior vena cava between the base of the heart and the diaphragm. This caused congestion of the portal venous system through the liver, pancreas and intestines. In all, about twenty-five adult white rats were used. One was used as a control without obstruction and in this instance the dye used circulated uniformly with the blood without evidence of emboli occluding arteries or arterioles. The total flow was obstructed in three rats — in the remainder the obstruction was partial.

TECHNIQUE

The white rats were anesthetized with sodium luminal.* A tracheotomy was done and the cannula of a small pulmotor was inserted and tied in place. The thoracic and abdominal viscera were exposed through a midline incision extending from the symphysis to the manubrium. Two lateral incisions were made, extending from

* 0.13 gm. subcutaneously is usually sufficient to anesthetize an adult rat. The animal is usually adequately anesthetized within thirty minutes.

THE CIRCULATION IN THE PANCREATIC LOBULE AFTER PARTIAL VENOUS OBSTRUCTION *

JAMES S. P. BECK, M.D., AND PAUL PETERSON, B.S.

*(From the Department of Medicine, Vanderbilt University School of Medicine,
Nashville, Tenn.)*

The object of the studies here reported was an analysis of the circulatory changes in the pancreatic lobule during venous obstruction. Little attention had been directed to the changes in the pancreas in chronic passive congestion until 1925, when VonGlahn and Chobot¹ described in detail for the first time the changes in the pancreas in cases of heart failure. Among the distinctive features observed were areas of capillary congestion at the periphery of the primary lobules, with no congestion of the vessels of the islands of Langerhans. The observations emphasized the importance of a careful study of the capillary patterns in these lobules.

Before 1925, however, DeWitt² had made a comprehensive study of the anatomy and physiology of the pancreas. Relative to the circulation she believed that the islands of Langerhans were supplied by venous channels arising from nearby veins. She found that there was stasis of blood in the island capillaries in chronic passive congestion and cites this in support of the venous origin of these vessels. Since this work, few investigations concerning the circulation in the pancreatic lobules have appeared.

Beck and Berg³ made an anatomical study of the circulatory pattern in the islands of Langerhans and found that the islands were located near the larger vessels of the lobules. They described short direct arterioles supplying the island capillaries, a free anastomosis between these capillaries and the interacinar capillaries, and short direct efferent venules draining the island network. Thus they were presented as units lying near the central vessels of the lobule, having a distinct and separate blood supply independent of the capillaries of the remainder of the lobule, except for the free anastomosis occurring between the insular network and the interalveolar rete. It

* Received for publication April 1, 1932.

THE CIRCULATION IN THE LOBULE WITH PARTIAL OBSTRUCTION OF THE INFERIOR VENA CAVA IMMEDIATELY PROXIMAL TO THE DIAPHRAGM

The peripheral capillaries were dilated and engorged with red blood corpuscles, while the arteries and arterioles were filled with black dye. The capillaries of the islands of Langerhans were well injected and stood out as black areas of tortuous capillaries (Fig. 2). There were occasionally seen other areas of partial injection, usually located near the islands. The short arterioles to the islands were blackened; the efferent venules were also, but to a lesser degree. In the long arterioles the dye was dense at the proximal end and a short distance along the course. The dye gradually faded before the peripheral capillaries were reached. The venules draining the peripheral capillaries were widened and engorged with red blood corpuscles and contained no dye. The arteries and arterioles in the lobules of the many pancreases examined were similar, but varied in dye distribution according to the amount of obstruction of the inferior vena cava. In every case in which the degree of obstruction was just short of being complete (the degree estimated at the time when the copper wire was placed around the inferior vena cava), there was an absence of dye in the periphery. In those cases in which the degree of obstruction was estimated as about one-half, a small amount of dye flowed into the peripheral capillaries. However, the capillaries of the islands contained dye in all degrees of obstruction with the single exception of complete obstruction.

THE CIRCULATION IN THE LOBULE FOLLOWING COMPLETE OBSTRUCTION OF THE INFERIOR VENA CAVA IMMEDIATELY PROXIMAL TO THE DIAPHRAGM

In these cases the intralobular arteries contained the black dye. The arterioles, both short and long, and the capillaries of both the islands and acinar tissue were free from it. The venules were markedly dilated and engorged with blood, as were the capillaries. The peripheral capillaries were more markedly dilated than those in the pancreatic islands (Fig. 3).

the subcostal angles and passing up the tenth intercostal space on each side toward the axillae. Such incisions usually avoid large arteries and veins so that hemorrhage is not troublesome. The four flaps formed by the incisions were retracted and held in place by clamps. This gave full exposure of both thoracic and abdominal viscera. Warm saline was used over the viscera to protect them from drying. The diaphragm was cut from its anterior and lateral attachments and the phrenic nerves were severed, thus paralyzing the diaphragm. By these procedures about 2 cm. of the inferior vena cava was exposed between the diaphragm and the heart. A ligature was placed loosely around the aorta just above the heart so that it could be tied immediately after the injection had been completed. By means of a soft copper wire placed around the inferior vena cava varied degrees of partial obstruction in some cases, and complete obstruction in others, were made. With the pulmotor working, the circulation could be kept going for periods sufficient to ensure definite enlargement of the liver and distention of the veins of the viscera. When the congestion was well marked the pericardium was opened and by means of a small hypodermic needle Higgins' commercial India ink was injected slowly into the left ventricle. The injection was continued until the liver became "peppered" with pinpoint black dots. The aorta was then tied and the animal immediately placed in cold 10 per cent formalin. Later the pancreas was dissected out and the lobules were teased apart and studied. Some lobules were sectioned with a razor blade and others were embedded in paraffin, sectioned and stained with hematoxylin and eosin. By far the best specimens were obtained by dehydrating, clearing and mounting whole lobules after they had been teased apart. They were cleared in methyl salicylate.

THE CIRCULATION IN THE LOBULE WITHOUT OBSTRUCTION

The capillaries of the lobules in these experiments were uniformly injected and there was no evidence of emboli obstructing the flow. The islands stood out as darker areas located near the larger vascular trunks (Fig. 1). These sections were used as controls.

The height of the fluid in the piezometer tubes, the speed of flow throughout the system, and how these are affected by varying degrees of obstruction at the outlet are more easily understood if the simplest possible condition exists. To begin with, therefore, the connecting-tube is omitted and the conditions examined in the simple U-tube. To observe the speed of flow in the system a dye may be injected through a rubber connection with a needle and syringe at any desired time. The rate at which the dye moves along indicates the speed of flow with accuracy sufficient for this experiment.

With a set level in the reservoir, and while the fluid is flowing freely through the system, the fluid level in P_1 is highest, and in P_6 it is the lowest. Between these points is a gradual uniform change in pressure, which, if charted, will form a straight line between the highest pressure and the lowest pressure. Thus is formed a uniform gradient of diminishing pressure along the length of the U-tube (see curve "a" on the chart). The pressure at any other point can be computed by hydraulic formulae. By partially occluding the outlet while the fluid is flowing, a change in pressure occurs at once throughout the system and is seen in the rising levels in the piezometer tubes. It is observed that the greatest change in pressure will be in the piezometer tube nearest the obstruction, and the least in the one nearest the reservoir. Between these points the change is intermediate. If the heights of the new levels (see curve "b" on the chart) are plotted on the previous chart the change is apparent. At once it is seen that a uniform diminishing gradient of pressure, caused by the obstruction, is formed between tubes P_6 and P_1 against the flow. The new gradient is formed at the expense of the gradient of pressure arising from the reservoir, and the speed of flow is lowered. As the obstruction is increased toward completeness the levels approach each other, the gradient becomes less and less, and the speed of flow becomes slower and slower. When the obstruction is complete, the levels are equal to each other and to that in the reservoir; there is no gradient and the flow is nil.

By inserting the connecting-tube to represent the circulation through the islands a slightly different set of conditions is produced. There is noted a slight change in the levels in the piezometer tubes when the flow through the tubes is established. There is a fall of the levels in the tubes of the proximal limb (see curve "x" on the chart). The greatest is in P_1 near the connecting-tube. The levels in the

PHYSICAL MODEL

The findings in these experiments were more easily interpreted when the pressure relationships were more accurately and critically examined in a physical model so constructed as to conform closely to the circulatory pattern in the pancreatic lobule.

Such an apparatus (Fig. 4) may be constructed with the use of a reservoir, a U-tube equipped with piezometer tubes, and suitable connections for incorporating a connecting-tube and constrictions. Each limb of the U-tube may conveniently be about 45 inches long with piezometer tubes erected at 15 inch intervals. The bend may also be 15 inches long. One limb (proximal) is attached to the lower end of the reservoir. The reservoir is of a type which maintains a constant level while the fluid is flowing through the system. In comparing the apparatus to the vascular system in the pancreatic lobule, the reservoir is the source of steady pressure and represents the intralobular artery. The proximal limb from the reservoir to the bend represents the small arterial branch arising from the intralobular artery which will carry blood to the peripheral parts of the lobule. The bend of the U-tube represents the peripheral capillaries, and the distal limb may represent the small vein which brings blood from the periphery back to the middle zone of the lobule. The circulation through the islands is represented by a connecting-tube (15 inches long) inserted into the system to join the proximal and distal limbs a short distance (about 15 inches) from the reservoir. The short arterial branch from the intralobular artery is represented by the segment of the proximal limb of the U-tube existing between the reservoir and the point of attachment of the connecting-tube; the insular capillaries are represented by the connecting-tube, and the short efferent veins are represented by the segment of the distal limb existing between the outlet of the system and the point of attachment of the connecting-tube.

The piezometer tubes are so placed as to indicate the pressure in the critical points of the system. Along the proximal limb the piezometer tube (P_1) is near the reservoir and adjacent to the connecting-tube, P_2 is between the connecting-tube and the bend, and P_3 is nearer the bend. Along the distal limb P_4 is near the bend, P_5 is between the bend and the point of communication made by the connecting-tube, and P_6 is adjacent to the connecting-tube.

there is again noted a sharper rise in pressure in the system near the obstruction. The rise becomes less and less as the reservoir is approached. The gradients are similar to those following obstruction in the previous experiment, with the exception of the slight alteration caused by the connecting-tube, the result being that there is greater slowing of the flow through the bend. The slowing is progressive as obstruction approaches completeness.

There are many islands, however, whose afferent arteries arise directly from the intralobular arteries instead of from the small arterial branch illustrated in the model. In this case the free anastomosis existing between the capillaries of the islands and those of the adjacent acinar tissue makes a similar relationship of pressure, though the amount of variation may be slightly less. The efferent venules are more commonly found as tributaries of a small vein which drains a part of the interacinar capillaries.

If a resistance like that of the capillaries of the periphery is now put into the system by means of a constriction in the U-tube at its bend, and a similar constriction is placed in the connecting-tube to represent the resistance offered by the insular network of capillaries, the apparatus more nearly represents the situation in the pancreatic lobules. The greater resistance of the interacinar capillaries may be represented by a constriction in the bend of the U-tube, which is narrower than the one in the connecting-tube which represents the slightly wider capillaries in the islands. As a result of these changes in the apparatus there is a marked alteration in the levels in the piezometer tubes. Those in the distal tube (P_6 , P_5 , P_4) are lowered somewhat, while those in the proximal tubes (P_1 , P_2 , P_3) are greatly elevated (see curve "y" on the chart). The explanation⁴ of these changes is simply the result of an adjustment of the pressures to the resistances placed in the apparatus. The less resistance in the connecting-tube will allow a greater volume of fluid to flow at a faster rate between the extremes of pressure. This will result in a still slower rate in flow through the smaller constriction at the bend, and is explained by the same principles above described when the capillaries of the U-tube and connecting-tube are uniform. If partial obstruction is applied at the outlet as before, there is, as a result, a much more marked slowing of the flow through the bend than was observed in any of the previous conditions. The slowing through the connecting-tube is not so marked. As obstruction approaches com-

tubes of the distal limb (P_4, P_5, P_6) are slightly elevated. The greatest change is in tube P_6 which is nearest the connecting-tube. The reason for these changes is readily seen when it is remembered that the connecting-tube allows a flow from the highest pressure of the proximal limb to the lowest pressure of the distal limb; it lies between the extremities of the gradient of pressure in the system. Thus a rapid flow is established, which allows a fall in pressure from the proximal limb and causes a slight rise in pressure in the distal limb as described. There follows a disturbance in the original uniformity

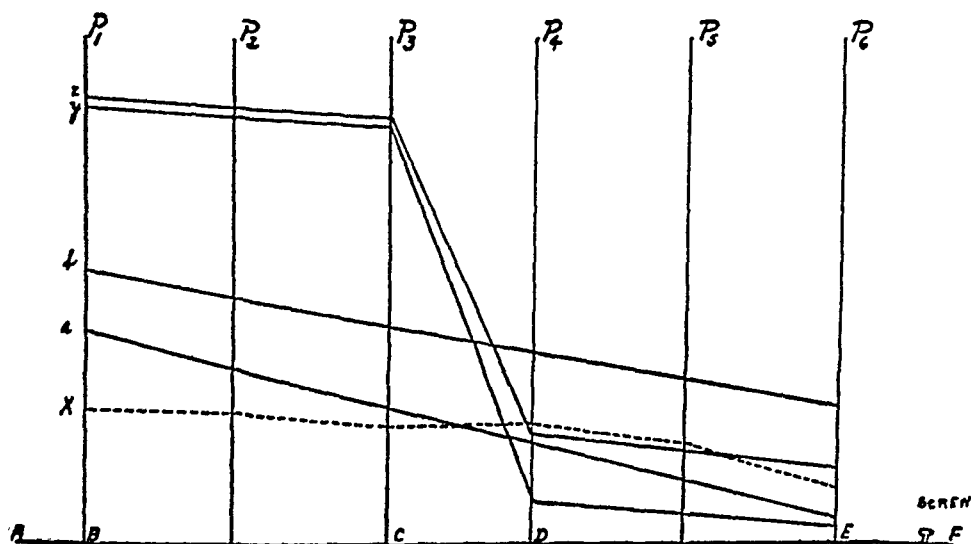


CHART 1

Curve "a" shows the gradual uniform change in pressure between piezometer tubes P_1 and P_6 with only the U-tube and no connections.

Curve "b" shows the uniform increase in pressure between tubes P_1 and P_6 , with partial occlusion at outlet in the U-tube with no connections.

Curve "x" represents the pressure after insertion of connecting-tube between the limbs of the U-tube.

Curve "y" represents the pressure in the tubes when the outlet has been partially obstructed and with the connecting-tube inserted.

Curve "z" represents the pressure when constrictions have been made in bend of U-tube and also in the connecting-tube, and with partial obstruction to the outlet.

of the gradient, which tends to approximate the pressure in the tubes P_3 and P_4 at the bend. At the same time there is less pressure to force the fluid along, as much of it is shunted through the connecting-tube. This results in a slowing of the flow through the bend.

If the outlet is again partially occluded while the fluid is flowing

structure of the circulatory pattern, which favors a faster flow through the islands of Langerhans than through the peripheral capillaries.

2. The flow through the islands is not seriously embarrassed in considerable venous obstruction, whereas in the peripheral capillaries there is a tendency to congestion.

3. Experiments with a simple physical model, which appear to parallel closely the animal experiments, indicate that the pressure relationships are the major factors involved.

NOTE: We wish to thank Dr. C. Sidney Burwell of the Department of Medicine for making possible these experiments and for helpful suggestions, Dr. Ernest W. Goodpasture of the Department of Pathology for reviewing some of the microscopic slides, and Drs. T. D. Cope and H. C. Barker of the Department of Physics of the University of Pennsylvania for helpful criticisms.

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DESCRIPTION OF PLATES

PLATE 104

- FIG. 1. Part of a pancreatic lobule. No venous obstruction. The arteries, veins and capillaries are uniformly filled with the black dye. Low power drawing.
- FIG. 2. The appearance of the pancreatic lobule in partial venous obstruction showing arteries, veins and islands of Langerhans. Low power drawing.

pleteness the rate of flow in the bend is so slowed that the movement of the fluid is barely perceptible, while that in the connecting-tube is readily perceived.

DISCUSSION

The findings from animal experimentation and observations made upon a physical model are in accordance with the conditions was observed in the peripheral and insular capillaries of the pancreas in cases of chronic passive congestion. The results of the animal experiments, though they clearly show a lack of dye in the peripheral capillaries in partial venous obstruction, do not indicate that the flow under these conditions is nil in these channels. The aorta was ligated immediately when the injection was considered complete, thus stopping the circulation completely. The injection was considered complete when the dye began to appear in the efferent venules of the enlarged liver — indicating that it had passed through some of the capillaries and had entered the portal venous system. The portal vein in each case was blackened. If time had been allowed, in any degree of partial obstruction, for the blood to travel through the peripheral capillaries, some dye undoubtedly would have been seen in these vessels. The experiments indicate, however, that there is a lack of uniformity of flow in all parts of the pancreatic lobule, which is due to the peculiar anatomical structure of the circulatory pattern. The result is that there is ensured a maximum flow of blood through the islands in various degrees of obstruction, at the same time favoring conditions for stasis in the capillaries of the marginal zones of the primary lobules.

In simplifying the physical model, the flexibility of the blood vessels, their tapering structure, the number of capillaries and branches, and their conformity in size were sacrificed. A fluid of little viscosity was used as the circulating medium. As the results of the experiments with the physical model appear to parallel closely those of the animal experiments, they indicate that the pressure relationships are the major factors involved.

SUMMARY

1. Animal experiments were done which indicate a lack of uniformity of the flow of blood in all parts of the primary lobules of the pancreas. This inequality of flow is based upon the anatomical

PLATE 105

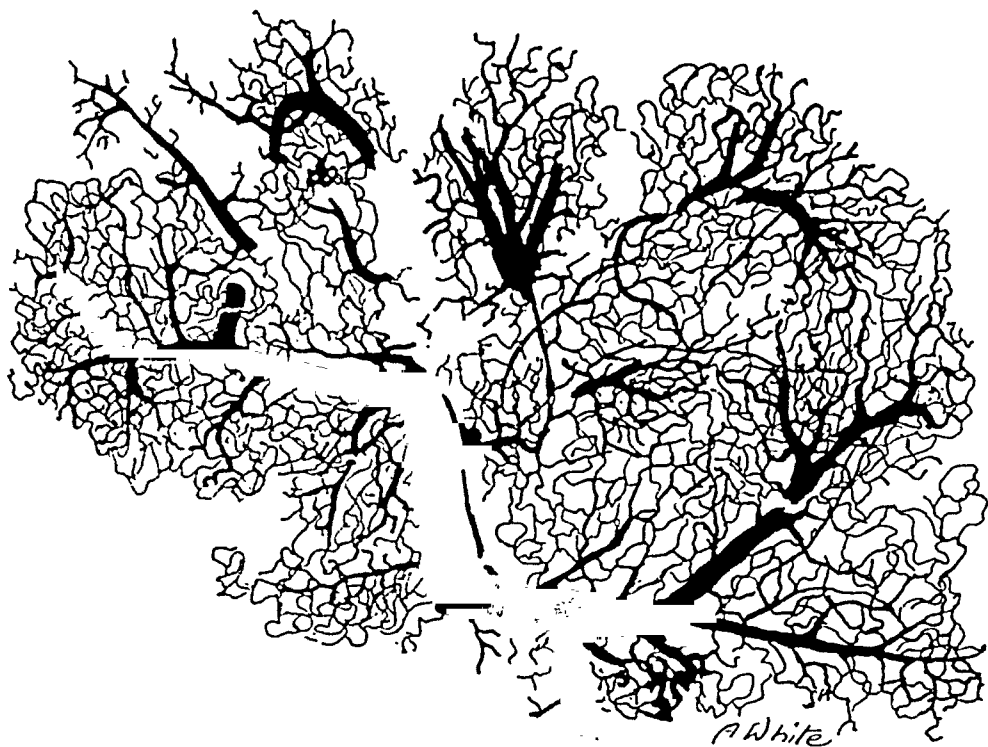
FIG. 2. The appearance of the lobule in complete venous obstruction showing acinar spaces and islands of Langerhans. Low power drawing.

FIG. 3. The model constructed to conform closely to the circulatory pattern of the acinar lobules.

CASTING MODEL	ANATOMICAL REPRESENTATION
A = reservoir	Intralobular artery
AB = last part of proximal limb of U-tube	Afferent arteriole to island of Langerhans
AC = proximal part of U-tube	Small artery to acinar tissue
CD = bend of U-tube	Interacinar capillaries
DE = distal limb of U-tube	Small vein accompanying small artery to acinar tissue
BE = connecting tube	Capillaries in islands
EF = last part of distal limb of U-tube	Efferent venule of islands
F = outlet of distal limb (screw to vary obstruction)	Intralobular vein

P₁, P₂, P₃, P₄, P₅, P₆, are piezometer tubes

(Compare with Figs. 1, 2 and 3.)



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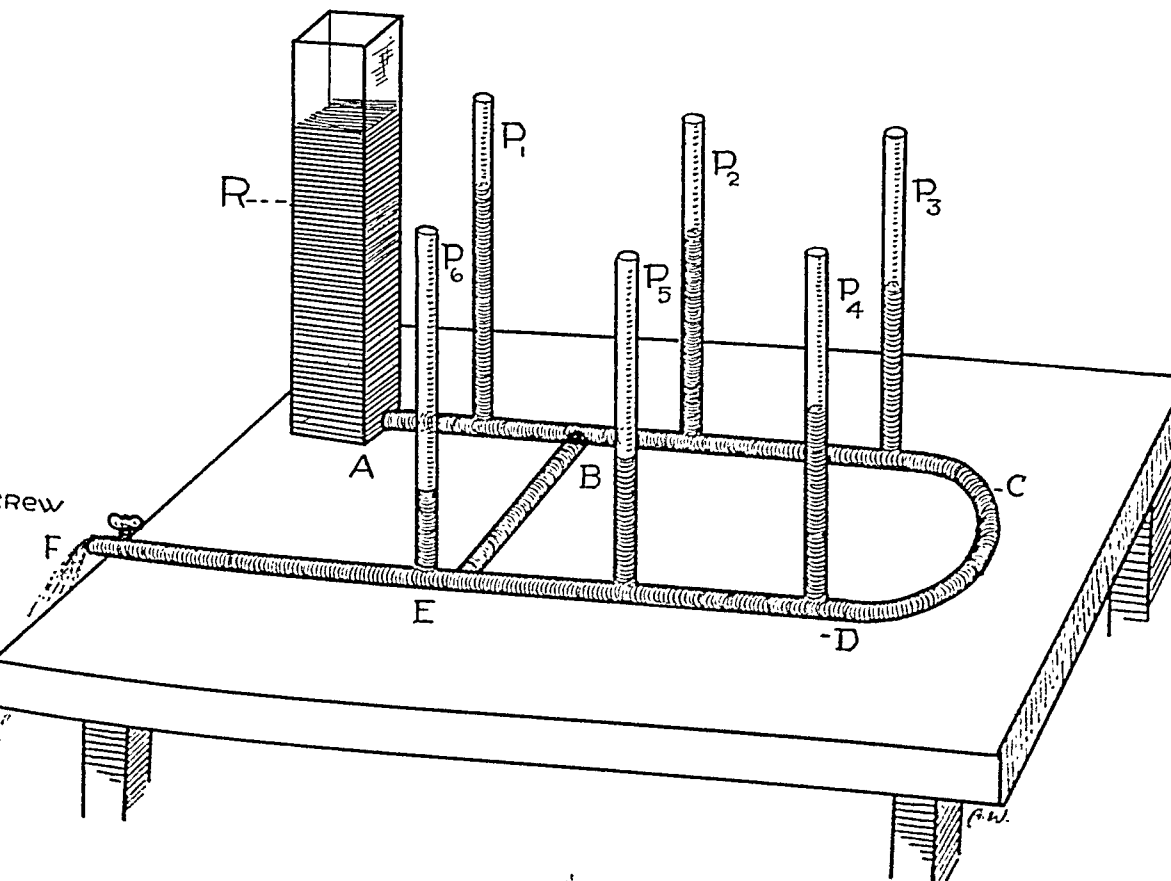


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Anna. C.



4

the undiluted stain and placed in the dish containing the diluted blood stain. After 2 to 10 minutes they were removed one by one, blotted (not dried), passed rapidly through two dishes of absolute alcohol into xylol, and then into clean xylol, from which they were mounted in balsam on a slide.

In order to compare the preparations stained with Wright's with those stained by other methods, some moist spreads were fixed in methyl alcohol and stained with Giemsa's stain; others were fixed in one-third absolute alcohol plus two-thirds saturated aqueous corrosive sublimate and stained with methyl green and acid fuchsin or with methyl blue and erythrosin; and some were fixed with Zenker-formol and stained with hematoxylin and eosin. All of the methods furnished excellent preparations, but the simple Wright's blood stain was the most satisfactory and furnished the most differentiation.

By means of Wright's stain the greatly hypertrophied spindle and stellate cells showed with marked clearness. The numerous chromatin granules in the large nuclei of these cells, described by Aragao,¹ were sharply stained a deep purple and the hypertrophied true nucleolus a pinkish blue. The myxomatous material described by Hobbs² in these cells was stained slightly pink. The granular inclusion body in the ectoderm cells, described by Rivers,³ was stained pink; the granules in the polymorphonuclear cells became a bright red, the basophilic granules in the mast cells a blackish purple; erythrocytes were orange; ingested red blood cells were a greenish blue and ingested polymorphonuclear cells showed as red granules and blue fragments of nuclei. Numerous rickettsia-like granules, probably the granules described by Lipschütz,⁴ present in epithelioid cells and in hypertrophied monocytes of the tumor were stained a pinkish purple.

By the Wright's blood stain it was evident that the greatly enlarged spindle and stellate connective cells, as described by Hyde and Gardner,⁵ with their hypertrophied hyperchromatic nuclei and myxomatous material were the cells that correspond to the malignant cells of mammalian tumors. It is probably from the presence of these cells that the lesion was designated as a myxoma, although the marked infiltration of polymorphonuclear leucocytes which takes place in the rabbit myxoma is not characteristic of tumors in general.

The ectoderm cells covering the lesion were not so clearly differentiated by the Wright's stain as by the method described by

A SIMPLE METHOD FOR STUDYING THE CYTOLOGY OF THE INFECTIOUS MYXOMA OF THE RABBIT *

MARGARET REED LEWIS AND RAYMOND E. GARDNER

*(From the Department of Embryology, Carnegie Institution of Washington, and
Department of Filterable Viruses, School of Hygiene, Johns Hopkins
University, Baltimore, Md.)*

It is well known that characteristic changes occur within host cells of animals and plants infected with certain filterable viruses. For this reason it seemed important to describe a simple and rapid method—in brief—a modified Wright's blood stain, that was found useful in studying the cells of the tumor produced by the virus of infectious myxomatosis of rabbits.

A small, covered Stender dish (about 1 inch high by $1\frac{1}{4}$ inches in diameter) was filled about half full with undiluted Wright's stain, and in another dish of the same size was placed about the same quantity of stain diluted one-half with distilled water. A tumor nodule of sufficient duration was selected and excised from the etherized animal. A thin slice of the tumor was laid flat on a thin 1 inch square coverglass and slowly dragged across its surface. This was quickly dropped, spread side down, into the dish of undiluted stain. It was important to avoid drying of the spread before it reached the stain, and yet to escape using tissue that was too moist. When the tumor was so moist that the cells did not stick to the coverglass, the piece of tumor was placed on filter paper until some of the serum drained away, or it was cut up with small curved scissors into a soft pulp and a little of this dragged across the coverglass. In order to obtain spreads of the ectoderm cells the outer layer of the epidermis was removed and cut up into a pulp with a fragment of moist, normal subcutaneous tissue taken from a nearby region.

A number of moist spreads were dropped into the same dish of stain where they were left from 1 to 10 minutes, or even longer, provided the dish of stain was covered to prevent evaporation. However, when it was not convenient to stain all of the spreads at once some of them were dropped into methyl alcohol where they were kept until needed. The coverglasses were removed one by one from

* Received for publication April 25, 1932.

trophied nuclei of the malignant cells became deep blue and the nucleolus bright red. Giemsa's stain was an excellent one, except that the ingested material, the granules of the polymorphonuclear leucocytes and the granules of the epithelioid cells, were all stained red and so not easily differentiated from one another.

In the earliest tumors studied (48 hours) neither the spindle cell nor the macrophage exhibited marked hypertrophy, although the nuclei of many of the spindle cells were already somewhat granular. Both types of cells frequently contained a number of purple granules so that they were not so strikingly different as in the larger tumors.

As the tumor increased in size the number of stellate cells (malignant cells) increased. These cells became much larger than the normal connective tissue cells, their nuclei hypertrophied and became granular and a diffuse staining material called myxomatous material became evident in the cytoplasm of a number of them. Most of these cells were free from the purple cytoplasmic granules, although even in the most advanced tumor examined occasionally a malignant cell was found with purple granules in the cytoplasm.

Coincident with the increase in size of the tumor there occurred an increase in number of monocytes, hypertrophied monocytes, macrophages and epithelioid cells. The epithelioid cells became larger and the number of granules became greatly increased, so that in the large tumors hypertrophied epithelioid cells with their cytoplasm filled with the small purple granules were frequent.

In an effort to gain an understanding of the nature of the small cytoplasmic granules many other types of tissue, including granulation tissue, rat and human tumors, and normal rat, rabbit and chicken skin, subcutaneous tissue, spleen, liver and lymph node, were studied by means of spreads fixed and stained with Wright's blood stain. While many monocytes, clasmatocytes and epithelioid cells were found in these tissues they did not exhibit the small purple granules characteristic of the infectious myxoma of the rabbit.

Just what the nature of the granules is has not been determined. They resemble bodies described as rickettsia in size, location in cells and in certain but not all staining reactions. Lipschütz claims to have determined that they are not rickettsia bodies. It is possible that they are accumulated protein material, arising from the infiltration of the tumor with myxomatous colloid, for Lewis⁸ found that chick embryo cells grown in a medium containing white of egg or

Rivers; nevertheless, the somewhat granular pink material lying at one side of the nucleus was readily identified.

Aside from the stellate cells (myxomatous cells) the most striking characteristic of the tumor tissue, differentiated by the Wright's stain, was the numerous, large, flat, granular epithelioid cells scattered through the dermal and hypodermal tissue, especially in the region of blood vessels. These cells contained many small granules stained pinkish purple that were quite different from the red granules of the polymorphonuclear cells, the blue granules of the basophiles and mast cells, or the greenish blue ingested material. The granules in the various epithelioid cells were of different sizes and shapes, although those present in one cell were in general about the same size. In a single cell they varied from small round granules and short rods to more or less triangular-shaped granules. They were massed around the centrosome at one side of the nucleus, from which they scattered out to more or less fill the cytoplasm of the cell. They exhibited appearances strikingly similar to the rickettsia bodies, *Dermocentroxenus Rickettsia* or *Rickettsia Prowazeki*, but were more like the body Cowdry⁶ described in heart water disease as *Rickettsia ruminantium*. These granules were present in cells of spreads prepared by the other methods described, but they were not so clearly differentiated by any of the other stains used as by the Wright's blood stain.

The cells which contained the granules belong to the group of hypertrophied and transformed mononuclear leucocyte (Lewis and Lewis⁷), and were usually of the epithelioid cell type, although the characteristically stained granules were also present in monocytes, hypertrophied monocytes, and macrophages. Other macrophages, containing ingested red blood cells and cellular debris, were often present in the same field. These cells contained some of the purple rickettsia-like granules, and the epithelioid cells occasionally had an ingested dead polymorphonuclear leucocyte, although as a rule the epithelioid cells did not contain ingested cellular debris.

While Wright's blood stain gave on the whole the most satisfactory results, some of the other methods differentiated some one structure even more clearly — for instance — with Wolbach's modification of Giemsa's stain the myxomatous material in the stellate cells appeared as a large pink body within the surrounding blue cytoplasm, and by Auerbach's method the chromatin granules in the hyper-

plasma became full of small, more or less even-sized granules called albumin granules. Hobbs suggested that the granular cells might be hypertrophied mast cells, but their differential staining with Wright's blood stain shows that the granules are not similar to those of the polymorphonuclear or of the basophilic leucocyte.

Whatever the nature of these bodies may be they were found to be characteristic of the many tumors of the infectious rabbit myxoma studied for a period of several years, and to be clearly demonstrated by means of moist spreads of the lesion fixed and stained by means of Wright's blood stain.

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SCIENTIFIC PROCEEDINGS OF THE
THIRTY-SECOND ANNUAL MEETING
OF THE
AMERICAN ASSOCIATION OF PATHOLOGISTS AND
BACTERIOLOGISTS

PHILADELPHIA, PENNSYLVANIA

April 28 and 29, 1932

Voted to adopt as the topic for the symposium for 1933 the subject of Pneumonia.

Voted that no papers on lymphatic tumors be accepted for publication in the American Journal of Pathology unless the tumors have been submitted to the Registry.

Voted to meet in Washington, D. C., on May 9 and 10, 1933, in conjunction with the meetings of the Congress of American Physicians and Surgeons.

THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

ABSTRACT OF BUSINESS SESSION

Voted to elect the following officers for 1932-1933:

<i>President</i>	E. T. BELL
<i>Vice-President</i>	O. T. AVERY
<i>Treasurer</i>	F. B. MALLORY
<i>Secretary</i>	HOWARD T. KARSNER
<i>Incoming Member of Council</i>	N. C. FOOT
<i>Assistant Secretary</i>	ROBERT A. MOORE

ABSTRACT OF MEETING OF THE COUNCIL

Voted to elect the following new members:

Donald C. Beaver	James Watson Kernohan
Virgil H. Cornell	Frank W. Konzelmann
Theodore J. Curphey	George F. Laidlaw
R. Philip Custer	Samuel A. Levinson
Vincent J. Dardinski	John Loesch
A. Hobson Davis	John Franklin Noble
Cornelia M. Downs	Gorton Ritchie
Marcos Fernan-Nunez	Andrea Saccone
Irving Graef	Tom Douglas Spies
Paul Henry Guttman	Evan Lee Stubbs
Charles H. Hitchcock	M. Juanita Thompson
Lloyd R. Jones	Leslie T. Webster
John W. Williams	

Voted to accept with regret the resignations of Dr. M. A. Barber and Dr. Channing Frothingham.

Voted to record with deep regret the deaths of Dr. C. G. Bull, Dr. V. A. Moore and Dr. A. S. Warthin.

A STUDY OF PATHOGEN-SELECTIVE CULTURES IN RELATION TO VACCINE THERAPY. Fred Boerner and Myer Solis-Cohen (by invitation), Philadelphia, Pa.

Abstract. The Solis-Cohen pathogen-selective method for preparing autogenous vaccines from mixed cultures is based upon the assumption that organisms capable of growing in the fresh, whole, coagulable blood of the patient are those which are most pathogenic for that individual. It consists of two simultaneous inoculations of the material to be cultured, one in a rich medium, such as Rose-now's brain broth, and the other in the patient's fresh, whole, coagulable blood *in vitro*. After a preliminary incubation of 24 hours, both cultures are examined and the organisms present in each are studied for identification. The organisms which appear in the blood are those selected to predominate in the vaccine. Four hundred pathogen-selective cultures from 150 patients were studied. In approximately one-third the results were identical in both the broth and the blood. In one-fourth only certain of the organisms present in the broth grew in the blood. In one-fourth none of the organisms present in the broth grew in the blood. In 11.5 per cent the organisms that grew in the blood failed to grow in the broth, and would therefore have been missed by the ordinary methods of culturing. Streptococci and staphylococci grew most frequently in the patient's blood, the hemolytic strains showing the highest percentage of the former and the aureus of the latter. Few of the Gram-negative cocci or of the non-pathogenic Gram-negative bacilli grew in the blood.

The pathogen-selective method of culturing has also been found useful as an aid in isolating such organisms as the streptococcus from mixed infections and contaminated material.

A STUDY OF BACTERIAL HYPERSENSITIVENESS, WITH SPECIAL REGARD TO ITS VALUE AS INDICATING PATHOGENICITY, AND WITH A COMPARISON OF CUTANEOUS, INTRACUTANEOUS AND SUBCUTANEOUS TESTS AND OF THEIR RELATIVE VALUES FOR SUGGESTING APPROPRIATE VACCINE DOSAGE. Myer Solis-Cohen (by invitation), Philadelphia, Pa.

Abstract. In a study of the reactions produced by intracutaneous injections of dead bacteria (with their soluble toxins) obtained from patients, those produced by organisms that grew in the patient's fresh, whole, coagulable blood *in vitro* were compared with those produced by organisms that were killed by the patient's blood. No correspondence was observed between ability to grow in the patient's blood and ability to produce a reaction when injected intracutaneously. It may be inferred therefore that the intracutaneous skin test does not differentiate organisms that are capable of infecting the patient from those against which he possesses good resistance, and consequently is not a reliable means of determining which organisms in a mixed culture should be included in a vaccine.

A comparison of simultaneous intracutaneous and cutaneous tests with the same vaccine showed both to be positive in 26 per cent of the cases, both to be negative in 10 per cent, and the intracutaneous to be positive and the cutaneous negative in 64 per cent, the former being 3 plus in 26 cases, 4 plus in 10, and 5 plus in 4. In no case was the cutaneous test positive and the corresponding intracutaneous test negative. The intracutaneous injection therefore furnishes a much more accurate test for hypersensitiveness than does the cutaneous inoculation, which moreover cannot be regarded as reliable.

AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

A PRELIMINARY REPORT ON THE EFFECT OF SHAKING AS APPLIED TO THE
VERNES TEST FOR SYPHILIS. Adelaide B. Baylis (by invitation), New York
City.

Abstract. In a series of 100 selected specimens of blood serum the classical flocculation test of Vernes was carried out and, at the same time, a duplicate test on the identical specimens by a modified technique, in which the tubes were mechanically shaken for 15 minutes immediately after the addition of the perythynol to the serum. The shaking was found to increase the sensitivity without evident danger of false positive reactions. Specimens giving a slight degree of flocculation in the classical test were found to give a more decisive result when shaken, some of them now giving no flocculation at all, and others a higher reading which could be regarded as more definitely positive in character. Shaking did not alter the readings on the control tubes in any instance.

The results indicate that physical agitation increases the opportunity for surface contact and reaction between the colloidal particles of the antigenic substance and the reagin of the serum, and suggest that analogous shaking may be of similar value in other serological reactions.

Discussion

(Dr. Reuben L. Kahn, Ann Arbor.) I was glad to hear the presentation by Miss Baylis on the problem of shaking in precipitation. When we first observed that shaking hastens the precipitation reaction in syphilis, we made quantitative studies of the effect of the speed and the duration of shaking on the reaction. We observed that when the shaking speed was excessively rapid or the duration too prolonged, there was a tendency for the precipitate to become emulsified, so that clear-cut particles would become indefinite. We observed in connection with our precipitation reaction that a 10 minute shaking period, for example, would often change a strongly positive reaction to a weak reaction. Since Miss Baylis speaks of shaking 15 minutes, it seems to me that perhaps a shorter period of time might give better results.

(Miss Baylis.) In these experiments I started shaking at a period of from 1 minute up to the entire period of 4 hours. From these preliminary studies, which are not reported, I was able to decide that shaking for 15 minutes gave a suitable reaction. After 15 minutes the reaction was rarely changed, and I also found in these tests that the shaking for 15 minutes sometimes served to change an otherwise indefinite result to a definite answer, either on the positive or the negative side.

(Dr. Kahn.) I may say that 15 minutes shaking from a practical point of view is a difficult problem. Some workers consider the 3 minute shaking period in our test as too lengthy.

(Miss Baylis, closing.) I may also add that I tried different speeds.

(Dr. Frank B. Lynch, Philadelphia.) I should like to ask if any comparative tests were run on the cultures, using normal blood instead of the patient's blood.

(Dr. Ward J. MacNeal, New York City.) I should like to remark that the suggestion of the use of a patient's blood as a culture medium to select from mixed material the organism pathogenic for that individual patient requires further evidence in order to support such a conclusive identification. I should like also to call attention to the fact that such a method is extremely helpful in attempting to obtain in cultures an organism which would otherwise be missed, and I think an outstanding example is Ducrey's bacillus of chancroid, of which it is extremely easy to obtain a culture by using the patient's blood or the bacteriologist's blood or sheep's blood for the primary culture, but it is extremely difficult to obtain a culture without blood.

(Dr. Solis-Cohen, closing.) First, in regard to the theory: about fifteen years ago in an endeavor to find out why the chicken and pigeon are immune to pneumococcic infection, while the mouse and rabbit are susceptible, the various known antibodies were studied by Heist, Solomon Solis-Cohen and Myer Solis-Cohen, to see whether they were present in the chicken and pigeon and not in the mouse and rabbit. It was found that the serum of a pigeon does not differ in its action upon pneumococci from the serum of a mouse or rabbit. In the whole, fresh, uncoagulable blood of the pigeon, however, a bactericidal factor was found present which is absent from the whole, coagulable blood of the mouse or rabbit. Later Matsunami and Kolmer, employing our method, found a similar bactericidal action against meningococci in the whole, coagulable blood of the resistant rabbit that was absent from the whole blood of the susceptible mouse. Similar parallels were observed between the varying susceptibility of laboratory animals to other organisms, with the formation of the hypothesis that when small numbers of bacteria are planted in fresh, uncoagulated blood, only those bacteria grow and multiply which are pathogenic for the species from which the blood is drawn. Upon this was based the assumption that organisms capable of growing in the fresh, whole, coagulable blood of an individual are those which are most pathogenic for him.

In regard to the therapeutic results, Lowe of Liverpool published 100 cases of rheumatism treated by this method, and others have reported excellent therapeutic results.

As regards therapeutic controls, the hospitals are full of cases treated with vaccine prepared in the ordinary manner.

As regards skin controls, the organisms were not tested on normal people, but on their own hosts.

Speaking about the pathogenic significance outside of the streptococci, I think this is illustrated very well in pyelitis and cystitis, conditions in which vaccine treatment is commonly of little value. Ordinarily the colon bacillus alone will grow in cultures from the urine, but when the urine is inoculated in the patient's whole, coagulable blood, in some cases the colon bacillus persists, while in other cases it disappears, and is replaced by streptococci or staphylococci, which also are present in cultures from the nose and throat of the same patient. In two of the cases charted the colon bacillus from the feces which grew in Rosenow's medium was killed off by the blood. This does not prove that the organisms growing in the blood are pathogenic, and the others are not, but it is very suggestive.

Reactions from intracutaneous and subcutaneous injections of the same vaccine were compared, those from the latter being divided into general, focal and local. The absence of general and focal reactions after intracutaneous injection makes one question the efficacy of this method for therapeutic administration. The intracutaneous injection was given as a test, prior to the therapeutic subcutaneous injection, the degree of reaction affording a guide in determining the appropriate initial therapeutic dose. In most cases the same dose was given intracutaneously and subcutaneously, but when the reaction from the former was very marked, a smaller amount was injected subcutaneously. In 61 per cent of the cases the reactions from the corresponding intracutaneous and subcutaneous injections were of equal intensity — in 13 per cent the intracutaneous was the more severe, and in 26 per cent the subcutaneous was the more severe. An initial therapeutic injection with dose based upon the reaction to a preceding intracutaneous test produced a reaction that was not severe in 87 per cent of the cases. The intracutaneous test may therefore be regarded as a fairly safe and accurate aid in determining the initial therapeutic dose, subsequent doses being satisfactorily determined according to the reaction produced by the immediately preceding subcutaneous injection.

Discussion

(Dr. Marcus W. Lyon, South Bend.) What are the therapeutic results obtained with these selective cultures?

(Dr. Solis-Cohen.) They are quite good; they are much better in cases that have the vaccine over a fairly long period of time — more than four or five doses. We have obtained good results in asthma, in rheumatism, and in sinus infections. The vaccine was often given before an operation on the sinus or tonsils, half the course before the operation and half afterwards, and the patients did much better than those who did not receive the vaccine. In pyelitis and in many other conditions the results were quite favorable.

(Dr. Max M. Strumia, Philadelphia.) Were any controls run in connection with the therapeutic applications, as well as the skin tests?

(Dr. E. T. Bell, Minneapolis.) I should like to have Dr. Solis-Cohen explain a little more fully the basis of the assumption that the organisms that grow in the blood are the ones responsible for infection. One would expect to find immune bodies in the blood that would inhibit the growth of the organism responsible for a chronic infection.

(Dr. E. C. Rosenow, Rochester, Minn.) I am rather puzzled over the large number of different varieties of organisms that are regarded as pathogenic and am wondering whether the method is considered sufficiently reliable to attach etiological significance to all of the various organisms that grow in the blood of any particular case, or only to some. The cause of different diseases is usually assumed to be one organism, and not a mixture. In our own work this assumption is borne out by the fact that with few exceptions only one organism, usually a streptococcus, localizes in animal tissues corresponding to those affected in the patient from whom isolated, following intravenous injection of primary cultures from various atria of infection. Specific vaccines prepared from the strains thus "proved guilty," when given in appropriate dosage, have been helpful in many cases.

Discussion

(Dr. B. J. Clawson, Minneapolis.) This work of Dr. Cannon's brought out two very fundamental considerations: the first, that the mechanism of holding organisms at the point of the allergic area is probably due to antibody rather than to a mechanical mechanism, and the second, that if vaccine is applied to diseases where we want a general immunity, the intravenous method should be used, rather than the intracutaneous or subcutaneous methods.

PHENOMENON OF LOCAL SKIN REACTIVITY TO BACTERIAL FILTRATES IN THE TREATMENT OF MOUSE SARCOMA 180. Gregory Schwartzman and (by invitation) Nicholas Michailovsky, New York City.

Abstract. The phenomenon of local skin reactivity to bacterial filtrates described by one of us (Schwartzman) in 1928 was later also reproduced in the liver, kidney (Schwartzman); testis, intestines, lymphatic glands, lungs, thymus, guinea pig liposarcoma (Gratia and Linz); stomach (Karsner, Ecker and Jackson); and knee joints (Moritz and Morley). It was elicited with a great variety of microorganisms (Schwartzman), and also with vaccine virus as the preparatory factor (Gratia and Linz). The animals in which the phenomenon was observed were rabbits, horses, goats (Schwartzman); and guinea pigs (Gratia and Linz). It could not be reproduced in mice and rats (Schwartzman). Assuming that malignant tumors may be of parasitic etiology (Gratia and Linz), it was thought that the hypothetical virus should then be capable of inducing a state of reactivity in the tumor tissue and thus render it susceptible to reacting factors in the blood stream. Five guinea pigs bearing liposarcoma were injected intravenously with *B. coli* culture filtrate. Two guinea pigs which died 24 hours later and two killed 48 hours later showed at autopsy hemorrhagic lesions in the tumor tissue and no lesions in other organs. The fifth guinea pig was left alive for further observations. Guinea pigs were selected because of their susceptibility to the phenomenon of local skin reactivity to bacterial filtrates.

Since it was deemed important to determine whether or not this phenomenon could be reproduced in transplantable tumors in mice the effect of bacterial filtrates upon Mouse Sarcoma 180 (Crocker Institute) was studied by the authors. This strain of sarcoma was selected on account of its high growth energy and malignancy. The bacterial filtrate employed was of high phenomenon-producing potency, as previously determined in rabbits (Schwartzman), namely "agar washings" filtrate of *Meningococcus* 44 D group I (*i. e.*, filtrate #1700 containing 1350 reacting units per cc.).

By means of single or repeated intravenous or intraperitoneal injections of this filtrate, it was possible to elicit prompt severe hemorrhage in Mouse Sarcoma 180. The first appearance of the effect resembled very closely the phenomenon of local skin reactivity to bacterial filtrates. The hemorrhage brought about progressive damage of the tumor, which was followed either by complete elimination of the tumor and healing in a high percentage of mice, or by a striking regression with further slow reappearance of tumor growth. The effect upon the tumor appeared to be selective, inasmuch as the intravenous and intraperitoneal injections of the filtrate produced no hemorrhagic lesions in other organs of the mouse.

Dr. Lynch asked if the work was done with normal persons as well as with these patients. I reported before this Association eleven years ago concerning large groups of normal persons who were tested with colon bacilli, streptococci and meningococci. The meningococcus from the spinal fluid of patients with cerebrospinal meningitis grew in most of the bloods of the people tested, but the meningococcus from the throats of carriers grew in only a few individuals, the conclusion being drawn that the spinal fluid strains of meningococci are much more virulent for man than are the carrier strains, and that the minority of men, whose blood permits the rapid growth of carrier strains, are more likely to develop meningitis after exposure to a carrier. Dr. Heist, who did the work, found that the carrier strains always grew in his own blood, which he used as a control. Before the paper was read, Dr. Heist died of meningococcic meningitis, despite all efforts to save him.

THE RELATION OF SENSITIZATION OF THE FLAGELLA AND SOMATA OF THE TYPHOID BACILLUS TO PHAGOCYTOSIS. Stuart Mudd, Balduin Lucké, and Max Strumia, Philadelphia, Pa.

Abstract. Antityphoid sera containing both somatic and flagellar agglutinins, and sera containing either only somatic, or only flagellar, agglutinins have been tested for their power to promote, *in vitro*, phagocytosis by macrophages and polymorphonuclear leucocytes. The somatic antisera were prepared by injection into rabbits of an aflagellate variant of the typhoid bacillus (strain 0901); the flagellar antisera were prepared by adsorption of the complete antisera with the aflagellate variant. All three types of sera were found capable of promoting phagocytosis of typhoid bacilli both by macrophages and by polymorphonuclear leucocytes. Digestion of the typhoid bacilli occurred rapidly within both types of phagocytes. Such digestion unless adequately taken into account may lead to fallacious results in phagocytosis studies. From this work it appears that the tendency in the literature to regard flagellar antibodies as of no value as defensive factors is unwarranted.

LOCAL IMMUNITY AND THE LOCAL FORMATION OF ANTIBODIES. Paul R. Cannon and (by invitation) F. L. Sullivan, Chicago, Ill.

Abstract. Evidence is submitted suggesting a correlation between locally increased resistance, local mobilization of cells of inflammation, particularly macrophages, and local formation of specific antibodies (agglutinins).

Rabbits were injected repeatedly intracutaneously in the same area with a formolized vaccine of *B. paratyphosus B*. At various intervals this area, a corresponding area on the opposite side, blood serum, liver, spleen, and other organs were extracted under comparable conditions with glycerol-saline solutions in order to determine the relative concentrations of agglutinin.

Specific agglutinins were found in relatively high concentration in the locally treated area before they could be detected in significant amounts in the blood serum or in other organs. Non-specific inflammation in the skin of the opposite side of an animal locally vaccinated did not lead to a concentration of antibodies in the inflamed area.

These findings tend to substantiate the view that a locally increased resistance may be obtained by measures which secure a local concentration of phagocytes and specific antibodies.

THERAPEUTIC APPLICATION OF BACTERIOPHAGE IN STAPHYLOCOCCUS BACTEREMIA. W. J. MacNeal and (by invitation) Frances C. Frisbee, New York City.

Abstract. A bacteriophage highly potent against staphylococci found in infections of the blood stream has been used for treatment of staphylococcus bacteremia, chiefly by intravenous injection, but also by local application to wounds and by subcutaneous injection. In a series of fifteen patients there have been eight deaths and seven recoveries. The treatment is not a simple matter, and the course of the disease leading to recovery is prolonged and beset with many dangers which may be fatal. The careful and intelligent use of bacteriophage may be expected to assist somewhat in the treatment of this very grave condition.

Discussion

(Dr. Max B. Lurie, Philadelphia.) Was the effect of the bacteriophage used in these cases tested on the staphylococci isolated from the blood of the patients?

(Dr. Reuben L. Kahn, Ann Arbor.) I might mention in this connection that we have had at the University of Michigan Hospital at Ann Arbor six cases of staphylococcus septicemia with multiple abscesses, three of which recovered following bacteriophage therapy. One did not completely recover; this was a girl 16 years old who developed osteomyelitis. We have also tried the use of bacteriophage in the treatment of osteomyelitis. A report based on ten cases is to appear soon. The conclusions of the surgeons seem to be that when the osteomyelitis is due to a pure culture of staphylococcus with freedom from other organisms, such as *B. pyocyaneus* and streptococci, the effect of bacteriophage is favorable.

Another point of interest is that in chronic cases of osteomyelitis (as shown by Albee) we have found bacteriophage in the wounds, especially with the Orr closed wound method of treatment. Some have expressed the likelihood that the therapeutic effect that Orr believes takes place as a result of his method of treatment might be due to the presence of bacteriophage. I think, as Dr. MacNeal pointed out, that while one cannot be over-enthusiastic about this method of treatment, yet, in a condition as severe as staphylococcus septicemia, especially with multiple abscesses, the use of bacteriophage would seem justifiable.

(Dr. Gregory Schwartzman, New York City.) Between 1923 and 1927, I was very much interested in the therapeutic application of bacteriophage. We divided our cases into two groups, those receiving the bacteriophage intravenously, and those receiving it locally. As Dr. MacNeal pointed out in his charts, the intravenous injections have very unpleasant effects. They invariably produce chills and rather severe shock. These effects may be attributed to the presence of a great deal of toxic substances in the bacteriophage culture.

I think that the outcome of the staphylococcus septicemia depends very much on the focus from which it originates. I agree with Dr. MacNeal that staphylococcus septicemia, following osteomyelitis, is usually fatal, but some staphylococcus septicemias originating from phlebitis may improve spontaneously. Some time ago, we had a case of extremely severe staphylococcus septicemia following a phlebitis of the uterine veins. The patient recovered, however, without any treatment.

Although there appeared to be a close resemblance between this reaction and the phenomenon of local skin reactivity to bacterial filtrates, it does not necessarily mean that a virus must be responsible for the state of reactivity of the tumor cells to reacting factors introduced via the blood stream. Studies on other possible explanations are on the way. Observations reported are considered of interest because there appears to be a remarkable selective destruction of a tumor which is of a high malignancy and of rapid growth, and which shows spontaneous regression only very rarely, and also because being obtained in mice, these observations offer an opportunity for further studies on the relation of the "phenomenon of local skin reactivity to bacterial filtrates" to problems of tumor growth.

Discussion

(Dr. Howard T. Karsner, Cleveland.) There is in this reaction a series of interesting changes demonstrated by the microscopic examination of tissues, which may perhaps throw some light upon the nature of the process. In our work on the rabbit stomach, and that of Dr. Moritz and his associates on the rabbit knee joints, we were impressed by the fact that the inflammation produced does not differ from the ordinary exudative variety of inflammation except that the amount of hemorrhage is striking in all situations. This leads one to suppose that damage to the blood vessels is severe. The anatomical demonstration of that damage to the blood vessels is difficult, but in the rabbit knee joint it is possible to show actual necrosis of the walls of the vessels. These examinations were made on animals treated in the regular way by injection under the skin, and then subsequently by intravenous injection. The first injection in the skin or other tissues produces of itself, apparently as a result of the irritative nature of the filtrate, a slight degree of inflammation, and this is markedly augmented when the intravenous injection is given. If the key part of the picture is damage to the blood vessels, — and whether that can be demonstrated morphologically after the first injection or not seems to be of little significance, — the fact that in some situations we can observe after the second injection actual damage to the blood vessels, and the further fact that hemorrhage is a prominent part of the reaction, leave no ground for doubt that vascular damage is severe, whether microscopically demonstrable or not. A sarcoma is a richly vascularized tumor, and it would seem, in these examples which Dr. Shwartzman has shown to us, that the damaging effect of the filtrate on the vessels has led to a profound hemorrhage, interference with the nutrition, and a consequent regression of the tumor. Since there is some interrelation between this reaction and the action of mocassin venom, the question naturally arises as to whether or not snake venom would have an effect upon the mouse sarcoma similar to that of the Shwartzman filtrates.

(Dr. Shwartzman, closing.) I only want to say in reply to Dr. Karsner that his suggestion to study the effect of snake venom is very interesting. However, the striking feature of the results reported is the selective effect of the meningococcus filtrate upon sarcoma 180, while the snake venom would presumably act as a general vascular poison. Of course, on the other hand, it is possible that the tumor blood vessels may prove to be more susceptible than the normal blood vessels to any vascular poison.

years ago, called attention to the influence of bacteriophage in the Orr treatment of osteomyelitis, and I think the bacteriophage has been used as a routine by Dr. Albee in the past three years; I thought it was unnecessary to mention that.

About the question of the shock, I think part of the shock may be due to foreign protein and on account of that we have eliminated the use of the broth preparation in intravenous work. We are now using a preparation which is almost completely protein-free. This does not give any shock in animals not infected with staphylococcus, and it does produce an effect in animals which are infected with staphylococcus. We are of the opinion that this agent is without a shocking effect on persons without a staphylococcus infection. A person who has a staphylococcus blood stream infection gets a shock. In our experience, one has to push the dosage of the phage to the point of shocking the patient to get favorable results. I do not think it is necessary to cause a chill for forty minutes; that is too long, but we feel our way carefully until we get a rise in temperature or a chill, or both, and then we pause to let the patient rest, but not too long, because the bacteria have usually not been eradicated, and we must begin with the phage again after a brief rest.

The question of the use of the other agents which were mentioned, foreign proteins: I must confess I would welcome any particulate agent that could be used in the treatment of bacteremias with such microorganisms as staphylococci and streptococci, and that would bring about a reaction from which recovery might result. Anyone who utilizes fatally stricken human beings as experimental animals (and that is what we are doing) gets a sort of clinical view in which he desires to get the patient well. I suppose that is unscientific, but if any one would show me that the injection of milk would get a person with bacteremia well, I would be glad to use it. In our experience four years ago we hoped that a coccemia would turn out to be a streptococcus bacteremia, because we thought there was some hope of the patient's recovering spontaneously, whereas with the staphylococcus we had very little hope. Now we hope it will be just the reverse. We think we have changed the prognosis a little bit. One of the prominent surgeons in New York, Charles Gordon Heyd, President of the State Medical Society, told me that the use of staphylococcus bacteriophage has altered the prognosis of postoperative infections.

CORNEAL REACTIONS TO BACTERIUM GRANULOSIS AND OTHER MICROÖRGANISMS. P. K. Olitsky, R. E. Knutti and (by invitation) J. R. Tyler, New York City.

Abstract. In an attempt to simulate in animals human trachomatous pannus, intracorneal inoculations of 14 varieties of bacteria and various other materials were made. The cornea of the rabbit was found to be highly sensitive to the action of injected bacteria. The lesions varied from insignificant transient changes to severe, destructive panophthalmitis. Animals that received the same organism showed like changes. Only *Bacterium granulosis* induced early, uncompleted and enduring lesions which resembled human trachomatous pannus. This effect was similar in rabbits and in monkeys.

Discussion

(Dr. E. C. Rosenow, Rochester, Minn.) I should be interested to know whether the culture of *B. granulosis* which had specific effects had been under

In view of the unpleasant consequences of intravenous bacteriophage therapy, we gave it up entirely and studied only the local effect of bacteriophage. Inasmuch as there is no bacteriophage for pyogenic streptococci, its application is limited only to staphylococcus and *B. coli* infections. The staphylococcus infections seem to yield quite well to the local application of bacteriophage. Pyelitis is another lesion which also seems to be influenced favorably by the bacteriophage if the treatment is kept up consistently for a certain length of time. There remains the question whether or not the effects obtained are due to the bacteriophage as such, or to the bacterial toxic substances included in the filtrates. I think that the local and general vaccination induced by the use of such filtrates plays a more important rôle than the lytic effect. In support of this point of view is the fact that in most instances cultures obtained from the bacteriophage treated wounds may grow quite profusely and prove to be resistant to the bacteriophage, in spite of the fact that the patients are doing better. One is more inclined, therefore, to the view that the effects are due either to the stimulation of the natural defense mechanism, or, as Dr. MacNeal thinks, to a change in the bacteria themselves, possibly in their antigenicity and pathogenicity.

(Dr. Preston Kyes, Chicago.) It seems to me that consideration of these clinical results following the intravenous injection of bacteriophage raises, at once, the question of the specificity of the reactions induced.

It is well established that the intravenous injection into man of particulate matter, or of reagents which induce the intravascular formation of particles, provokes the type of chill reaction seen in the cases reported. It is also recognized that when such non-specific reactions are provoked, they may be attended by a profound modification of an existing infection. I see nothing in the results reported in this particular paper which has not been claimed by those who have used intravenous injections of milk, of raw serum, of peptone, of suspensions of killed heterologous bacteria, and so on in the non-specific treatment of generalized infections.

Without questioning the favorable clinical results in the group of cases presented, I would question the deduction that the action of the bacteriophage in these cases is shown to be that of a specific reagent.

(Dr. MacNeal, closing.) Obviously the paper was considerably abbreviated in presentation, and I hope you will take that into consideration.

In regard to the assumption of specificity of the bacteriolytic agent for the bacteria, that was not based on the behavior of the patient, but on test tube experiments. In each instance a culture of the staphylococcus was treated by the bacteriophage, and the culture was completely sterilized by it in the test tube, so we have evidence that there is some reaction between the bacteriophage and the microbe. It seems to me that nobody who is familiar with the work of Gratia can doubt that there is an agent effective against staphylococci. I had assumed I could pass over that. We have insisted in each instance that a culture should be tested out against bacteriophage in the laboratory, and so far, in staphylococcus bacteremias, we have not come across one which is not susceptible to that agent. The thing I wish to discount is the assumption that such a phenomenon necessarily occurs also in the human body. I do believe, however, that some part of this influence may remain active in the body of the patient.

In regard to the osteomyelitis cases, I may say I purposely omitted any discussion of chronic osteomyelitis. I think Miss Patterson and Dr. Albee, some

show that the membrane contains no fibrin. A high percentage of the membranes studied contain fat in large amounts. It was possible to produce characteristic "hyaline" membranes in the lungs of dead animals subjected to forceful artificial respiration while autolyzed purulent material was being injected into the trachea. From histological and experimental studies, it is concluded that the membrane is formed by the mechanical action of forcefully inspired air upon a viscous material in the air spaces. The viscous material is pressed against the alveolar walls in membrane formation and surrounds bubbles of entrapped air. Under similar mechanical conditions of dyspnea in the presence of semifluid material in the air passages, membranes identical in form and position, but not in staining reactions, may be found in a variety of circumstances. The composition of such membranes depends upon the material which happens to be in the air spaces at the time. The term *dyspneal* membrane is suggested as the most descriptive and appropriate designation for this histological picture.

Discussion

(Dr. George R. Callender, Washington.) I should like to ask Dr. Farber whether he found that the epithelial lining of the alveoli was or was not desquamated beneath this membrane.

(Dr. Farber.) We have found necrotic alveolar walls with desquamated epithelium in some cases, but more often the walls were intact.

(Dr. Callender.) The reason I asked was that I felt in going over the influenza lungs and also the lungs of ordinary pneumonia cases from the war period that early there was a rather thin exudate, and then following the desquamation of epithelium, a denser exudate was thrown out. I have also had some of the inspiration cases in babies, and in those occasionally have found small strands of fibrin, but as a whole, they were like those you have shown.

(Dr. Farber, closing.) We have occasionally seen small strands of fibrin superimposed on the membrane, but in those cases there was also a superimposed exudate.

CATAPHORETIC VELOCITY OF STREPTOCOCCI AND PNEUMOCOCCI AS ISOLATED IN STUDIES OF ACUTE COLDS, INFLUENZA AND PNEUMONIA. Edward C. Rose- now, Rochester, Minn.

Abstract. The cataphoretic velocities of the streptococci and pneumococci isolated from nasopharynx, tonsils or sputum were determined by direct observation under the microscope, in an electrical field of constant voltage, by noting the time required to traverse the unit distance of 50 microns, and are expressed in terms of microns per second, per volt per centimeter.

The cataphoretic velocities of the streptococci or pneumococci, as isolated from the different groups, were determined over an extended period, before, during, and after an epidemic of influenza, and were found to vary roughly according to the different forms of infection of the respiratory tract. The streptococci or pneumococci isolated from the nasopharynx in cases of acute rhinitis had a velocity chiefly of about 2.56 (rhinotropic); those from cases of acute pharyngitis chiefly of about 1.83 (pharyngotropic); those from cases of influenza and influenzal pneumonia chiefly of about 1.83, 1.60, 1.42 and 1.28 (influenzal and bronchotropic); and those from lobar pneumonia or croupous pneumonia chiefly of about 3.91 (pneumotropic). During the epidemic of influenza a large

cultivation on artificial media for a long time or whether it had been isolated shortly before from experimental animals.

(Dr. N. W. Popoff, Rochester, N. Y.) In connection with the work of Watanabe on non-specific inclusions produced by various substances I should like to ask whether controls with heat-killed bacteria, bacterial filtrates, other bacterial toxins and human serum were used in his experiments.

(Dr. Knutti, closing.) In answer to the question about toxins, no toxin has been demonstrated for *B. granulosis* itself. There were no filtrates of *B. granulosis* used; only the heat-killed organisms were injected intracorneally.

In regard to Dr. Rosenow's question, as to the source and age of these cultures: some of the cultures had been isolated from cases of trachoma up to two years before inoculation; others were fresh cultures. No difference in effect was discernible when old or young strains were injected.

REPORT OF THE LYMPHATIC TUMOR REGISTRY FOR THE YEAR 1931. George R. Callender, Washington, D. C.

Abstract. During the first four years, 1925-1928, inclusive, 100 cases were received; during the fifth year, 50 cases; during the sixth year, 85 cases, and during the last, or seventh year (1931), 60, making the present total 295 cases.

Of this group of 295, 50 are not lymphatic tumors. A considerable number of the cases are not entirely suitable for study because either inadequate material, tissues and blood smears, or inadequate data, have been furnished. Approximately 65 per cent are of some value for study, but only about 50 per cent of the total number have sufficiently complete records and adequate material. Because of poor follow-up, the most valuable cases from the standpoint of the Registry have been completed cases in which history is adequate, autopsy has been done and material furnished from the biopsy, autopsy, or both. Information with reference to radiation treatment, its kind and effect, is very meager. What few data have been accumulated indicate that the reaction of the tumor to radiation is of considerable importance.

It is believed that the members of this Association can find many cases in their files which are complete and the Committee urges that as many of such completed cases be sent to the Registry as possible, irrespective of whether they offer difficulties in diagnosis or not. The Committee further recommends that no case or cases of lymphatic tumors be accepted for publication in the American Journal of Pathology unless these cases have been placed in the Lymphatic Tumor Registry. A study of certain groups of the tumors is presented as a part of this report. It will be published in a later number of the Journal.

ON THE NATURE OF THE "HYALINE" MEMBRANE IN THE LUNGS. Sidney Farber, Boston, Mass.

Abstract. A study was made of the occurrence, composition, and mechanism of formation of the hyaline membrane, which has been generally considered pathognomonic of influenzal pneumonia. Membranes, indistinguishable in appearance and staining reactions from those seen in influenza, were repeatedly found in the lungs of young children, usually in association with streptococcus bronchopneumonia. No evidence of influenza could be found in these cases. Identical membranes were seen in the mediastinum in the presence of mediastinal emphysema and mediastinitis, and in the lungs of newborn infants who had aspirated amniotic sac contents after intrauterine asphyxia. Staining reactions

pig or rabbit after death. The dead animals were kept at room temperature (25 to 30° C) for periods of from 5 to 48 hours before autopsying and culturing. The temperature of the morgue (40° F) in which the human bodies are kept after death prevented invasion of these organisms throughout the animal body even after 72 to 96 hours. An analysis of our observations made upon human necropsies indicates that there is no significant difference in the bacteriological findings of necropsies cultured within 9 to 48 hours after death from those cultured within the first 4 hours. Therefore, postmortem invasion is not a factor in accounting for the presence of bacteria within the organs and body fluids in this group of necropsies.

Our observations reveal that certain organs contain a greater frequency of bacteria than do other organs. It is observed that cultures from the liver and kidneys contain a significantly greater number of bacteria than cultures obtained from heart blood. Heart blood cultures alone are not a true indication of the bacteriological flora of the body at necropsy. Postmortem cultures of the lungs are of little value unless there is a structural alteration that is associated with a known type of bacteria.

Further analysis of the data is in progress and will be reported in full at a later time.

Discussion

(Dr. Ward J. MacNeal, New York City.) Your icebox temperature was 40° Fahrenheit?

(Dr. Burn.) Yes.

(Dr. M. A. Kugel, New York City.) About three years ago Dr. E. Z. Epstein and I became interested in the significance of postmortem bacteriology. This study was carried out under the direction of Dr. Gregory Schwartzman. Our series was not as large as that of Dr. Burn. We examined at postmortem the blood from the inferior vena cava, the bone marrow, the heart muscle and heart valves in sixty-six cases, and were surprised at the high frequency with which organisms were recovered from these sources. This was contrary to previous findings by other workers, but the work of Dr. Burn bears out most of our original observations.

Our solid media cultures showed little or no growth. However, the material in enriched fluid media aerobically and anaerobically usually gave good results. On the basis of our studies we came to the following conclusions:

1. That no significance can be attached to the recovery at necropsy of such organisms as streptococcus alpha, streptococcus gamma, enterococcus, staphylococcus aureus, coli and pyocyaneus bacilli, unless the same organism has been recovered during life.

2. When we found unusual organisms at postmortem examination, they were of significance.

3. If we had an individual who, during life, had a specific infection, such as pneumonia, and so on, we usually recovered the infecting agent in pure culture at postmortem.

(Dr. N. W. Popoff, Rochester, N. Y.) How long do you keep your cultures before you consider them negative? From the experiments by Truffi on the development of pathologic organisms in dead tissues it is evident that both artificial and natural immunization against particular microorganisms prevents or retards the growth of such organisms in tissues, and that it is only after destruc-

proportion of normal persons and patients suffering from chronic systemic diseases became carriers of streptococci having influenzal or bronchotropic velocity, whether exposed to influenza or not.

THE PRODUCTION OF THE "G" TYPE COLONIES OF *C. DIPHTHERIAE*, PARK NO. 8 STRAIN. Harry E. Morton (by invitation), Philadelphia, Pa.

Abstract. Organisms resembling *C. diphtheriae* in morphology were obtained from Berkefeld or Chamberlain filtrates of the Park 8 strain by (1) cultivation of diphtheria toxin (confirming Hauduroy's work); (2) allowing broth cultures to evaporate to dryness, redissolving in sterile distilled water and filtering (confirming the work of Smith and Jordan); (3) forced dissociation by the presence of LiCl in the culture media (the method employed by Hadley and Richardson); and (4) serial passage in acid broth.

The "G" colonies, which are the first visible colonies obtained during the cultivation of the filterable form, may be visible only by means of the low power objective of the microscope or a hand lens magnifying fourteen times. They are at first made up of extremely short rods, some Gram-positive, some Gram-negative, and some Gram-negative containing Gram-positive granules. Upon continual cultivation on suitable media more and more of the rods became Gram-positive, increased in size to that of the normal diphtheria bacillus, and assumed the various characteristic involution forms and groupings. Fermentation reactions of the "G" forms varied greatly. The only suggestion of virulence was the development of paralysis in guinea pigs in about one month after inoculation with "G" type culture.

Discussion

(Dr. Joseph D. Aronson, Philadelphia.) I should like to ask whether or not any neutralization experiments with diphtheria antitoxin were carried out with this organism.

(Dr. Morton, closing.) No antitoxin was added to any of these emulsions.

OBSERVATIONS CONCERNING POSTMORTEM BACTERIOLOGY. Casper G. Burn (by invitation), New Haven, Conn.

Abstract. An investigation has been in progress during the past two years concerning the postmortem flora of the human body. Up to the present time there are no data available which permit an evaluation of postmortem bacteriological findings, either from the point of view of general biological interest, or of possible correlation of ante mortem bacteriology.

This report is a study based upon observations made from 300 unselected necropsies. Both aerobic and anaerobic methods were employed for cultivation of bacteria from the organs and body fluids.

The results indicate that the organs at necropsy contain a high frequency of bacteria. The strains of bacteria isolated consisted of a variety of potentially pathogenic organisms. *B. coli*, staphylococci and streptococci were the predominating groups of bacteria isolated from the organs. Since these organisms are common inhabitants of either the respiratory or intestinal tract, they may be considered as postmortem invaders. Experimental studies pertaining to this phase of the problem indicate that *B. Welchii*, *B. coli* and staphylococci were the only bacteria that successfully invaded throughout the body of the guinea

The physiological effects on the hematopoietic organs from acute infection, therefore, seemed to be the opposite of the action of liver extract, and this may possibly explain the inhibitory effect of infection on the action of the material effective in pernicious anemia.

FURTHER STUDIES ON EXPERIMENTAL LEPROSY AND THE CULTIVATION OF *B. LEPRÆ*. Earl B. McKinley, Washington, D. C., and Malcolm H. Soule, Ann Arbor, Mich.

Abstract. Recent work on the cultivation of *Mycobacterium leprae* and the production of experimental lesions in monkeys with both fresh leprosy tissue emulsion and cultures of the organism isolated from leprosy tissue is reviewed. Since previous reports the cultures of *M. leprae* have been carried through a total of twelve generations and are now in the thirteenth. The organism has been under artificial cultivation for over one year and has shown no tendency to grow with greater ease, but on the contrary is growing with increasing difficulty. A hormone glycerol agar has continued to be the most satisfactory medium for growth of the leprosy bacillus, and a gaseous tension of 10 per cent carbon dioxide with 40 per cent oxygen has proved most satisfactory. Various vegetable and amino-acid mediums have been employed without success. Cultures from the ninth, tenth, and eleventh generations have been tested by intradermal inoculation in older monkeys than formerly employed, and either these animals are resistant to the infection or our cultures have lost in virulence on artificial media. Serological data dealing with complement fixation and agglutination are presented, as well as a discussion concerning the mechanism of infection in leprosy in human beings and under experimental conditions.

Discussion

(Dr. Joseph D. Aronson, Philadelphia.) In association with the late Dr. Paul A. Lewis a serological study was carried out on the serum of leprosy patients hospitalized at Carville, La. We found that the serum from these patients gave a positive complement fixation reaction with antigens prepared from the Duval, Kedrowsky and the Clegg strain of *M. leprae*. On the other hand, serum from tuberculous individuals, as well as from tuberculous guinea pigs and goats, reacted only with antigens prepared from the Duval and from the Kedrowsky strains, which are non-chromogenic and resemble culturally the avian type of tubercle bacilli, but failed to react with the Clegg strain, which is chromogenic. By means of absorption experiments Dr. Furth showed that the non-chromogenic Duval and Kedrowsky strains were antigenically identical with the avian type of tubercle bacillus. Since that time we have found that tuberculin prepared from these strains produces a positive tuberculin reaction when injected into the wattle of tuberculous chickens, and in tissue culture the growth of spleen and bone marrow from tuberculous chickens is inhibited; whereas tuberculin prepared from the Clegg chromogenic strain fails to produce a tuberculin reaction in tuberculous chickens and does not inhibit the growth of explants from tuberculous chickens. I should like to know how the culture isolated by Drs. McKinley and Soule differs from the Duval and Kedrowsky strains.

(Dr. McKinley, closing.) As I said, our experience on the serological side is very limited. We have not done any more work than is indicated in our report to-day. We have not compared this strain serologically with the Duval or

tion of antagonistic substances that the microorganisms begin to grow without restriction. Have you noticed in your studies any difference as to the time of appearance and character of the growth in cadaverous tissues from individuals with a history of naturally or artificially acquired immunity toward particular organisms?

(Dr. Edwin F. Hirsch, Chicago.) In the routine examination of brains it is common to find multiple cystic cavities, and we look upon these from the practical standpoint as due to postmortem invasion of the brain by gas-producing bacteria, and do not attach particular significance to them. Is this Dr. Burn's experience?

(Dr. Joseph Aronson, Philadelphia.) I should like to ask Dr. Burn whether there is a relation between the bacterial flora and the pathological changes in the organs.

(Dr. Burn, closing.) Concerning the length of time that we retain cultures, we always keep them at least two weeks before discarding. Due to the large number of cultures made at each necropsy and the frequency of necropsies, it is essential to discard them for the purpose of obtaining incubator space.

Since our studies are incomplete in many ways, we cannot answer directly the question pertaining to antibodies in the organ at necropsy.

In regard to the relation of postmortem invasion to cysts found in the brain at necropsy, we have recently cultured twenty brains; but have found bacteria present in only four instances. The strains isolated were chiefly streptococci.

We have very frequently found bacteria in the organs without any evidence of histological changes within the organs. However, further study of our material will be necessary before anything definite can be said about this.

RETICULOCYTES AND BONE MARROW CHANGES IN PIGEONS AFTER INFECTION AND THE ADMINISTRATION OF LIVER EXTRACT. Gulli Lindh Muller (by invitation), Boston, Mass.

Abstract. To obtain some possible explanation for the failure of an adequate reticulocyte response and improvement after the administration of substances effective in pernicious anemia, to pernicious anemia patients suffering from infection, the following experiments have been carried out on pigeons, an animal peculiarly susceptible to the potent material. Groups of pigeons under standard laboratory conditions were given (a) staphylococcus aureus infection intramuscularly, (b) liver extract 343 (N. N. R.) by mouth, and (c) both infection and liver extract.

After infection alone there was, as a rule, during the height of the reaction, a drop in the reticulocytes from the normal level followed by an increase above normal during the period of convalescence. Active mitosis was observed in the red as well as the white blood cells of the bone marrow after 24 to 48 hours, and about the fifth to seventh day the red blood cell centers had increased enormously, indicating that infection, directly or indirectly, stimulates red blood cell formation.

Normal pigeons fed liver extract showed a characteristic reticulocyte response, but the bone marrow showed no extension of hematopoietic tissue, rather a decrease of hematopoietic activity as evidenced by a decrease of mitotic figures and a diminution in the number of megaloblasts, features observed also in patients with pernicious anemia after liver therapy.

Infection combined with the administration of liver extract resulted in a mixed reaction with the features of infection predominating.

cattle in various parts of the state. Almost all of the cultures from patients with undulant fever, as well as those from cattle, were found to have the characteristics generally attributed to bovine strains.

Discussion

(Dr. E. M. Medlar, Mt. McGregor.) We have carried on for the past four or five years a rather extensive study of *B. abortus* infection in our dairy herd. We have also carried on a careful study in an attempt to increase the virulence of cultures we have in the laboratory. Some of these cultures have behaved very peculiarly in that on an increase in virulence there has been a change in their agglutinability. I would like to ask the question whether it is possible at the present time to establish a clearcut distinction between the three types of *B. abortus*, the human, the porcine and the bovine. We are of the opinion that it is extremely difficult. I wonder if the change may not be due to a change in the environment, either *in vitro* in the culture media, or *in vivo* in the cattle, pigs or human beings infected. Might this not bring about a change in the type of organism, so that we cannot say this is a porcine type, and this is a bovine type?

(Miss Coleman, closing.) No attempt has been made to show that these types can be completely differentiated. Apparently it is very difficult to do so. The point which seems to be of particular interest is that practically all the strains we have isolated, whether from patients' blood or cows' milk, have behaved similarly. Also, in so far as could be determined, they have the characteristics which have been attributed to the bovine type. Whether or not the microorganisms of the abortus-melitensis group can be definitely divided into distinct types or subspecies is, I believe, questionable.

MELITENSIS MENINGO-ENCEPHALITIS. G. H. Hansmann and (by invitation) J. R. Schenken, Iowa City, Ia.

Abstract. The communication concerns a review of the localization of the Brucella group of organisms in the central nervous system. An organism of this group has been isolated from the spinal fluid of four cases. We have found no reference other than ours which deals with a complete postmortem examination. The localization may be a complication of a well marked clinical case of Malta fever, or it may be the only manifestation of the disease. Agglutinins may or may not be present in the blood. A mononuclear pleocytosis usually of less than 200 cells is an outstanding feature of the spinal fluid. Startling evanescent central nervous system symptoms such as diplopia, hemiplegia, paraplegia, convulsions, and so on, are common. Two of thirteen cases have proved fatal. The organisms may be regularly isolated from the spinal fluid if 10 cc. of fluid be used as an inoculum. The organism in our case was of the porcine variety. The pathological changes were diffuse chronic meningo-encephalitis with a diffuse arteritis at the base of the brain, which led to a mycotic aneurysm of the basilar artery. A rupture of this aneurysm resulted in death.

Discussion

(Dr. C. A. Pons, by invitation, Asbury Park, N. J.) I want to report one case of *B. abortus* meningitis observed last July in Asbury Park. There had been an outbreak of undulant fever from a dairy. The total number of cases

Kedrowsky strains. We have had many cultures of supposed *B. leprae* in the laboratory, but I cannot answer Dr. Aronson's question on the basis of any specific differences antigenically.

I should also like to mention that Dr. Wade, who was to give the next paper, and who is unable to be here, has brought from Vienna some chromogenic acidfast bacilli which Lowenstein discovered accidentally in his blood work in tuberculosis. Out of about 1000 cases he has seven which are acid-fast and chromogenic. This is the first instance I know of where a chromogenic acid-fast organism of the type described in leprosy has been isolated from non-leprous human beings, and he has seven of them. I told Dr. Wade I would mention this work to-day.

A COMPARISON OF TYPHUS AND SPOTTED FEVER RICKETTSIAE IN TISSUE CULTURES. Henry Pinkerton and (by invitation) G. M. Hass, Boston, Mass.

Abstract. By incubating infected tissue cultures at 32° C voluminous multiplication of both typhus and spotted fever Rickettsiae was obtained.

Typhus Rickettsiae completely fill the cytoplasm of cells which they infect. The infection spreads rapidly and by 14 to 21 days has involved practically every cell in the cultures. Large colony-like masses of organisms are found regularly in these typhus cultures. The organisms multiply only within cells, however, and the colony-like masses are found on careful study to be greatly enlarged cells distended with Rickettsiae. This condition remains constant as long as successful cultures of the cells can be maintained (up to 52 days in these experiments). Typhus Rickettsiae do not invade the nuclei of cells.

A mild strain of spotted fever (the Reimann strain) has been studied by the same technique. In this disease also the majority of cells in the cultures become infected and the organisms multiply only within cells. Morphologically and tinctorially, spotted fever Rickettsiae differ only slightly from typhus Rickettsiae. Spotted fever Rickettsiae, however, infect the cytoplasm of cells only sparsely and irregularly, but multiply massively within nuclei. Similar intranuclear massing occurs in cultures of Rumreich's eastern spotted fever. The intranuclear multiplication corresponds to that described by Wolbach in ticks infected with spotted fever, but has not previously been seen in mammalian tissues. The intranuclear clusters of spotted fever Rickettsiae are compared and contrasted with "inclusion bodies."

COMPARISON OF THE INCITANTS OF UNDULANT FEVER IN MAN AND CONTAGIOUS ABORTION IN CATTLE IN NEW YORK STATE. Ruth Gilbert and (by invitation) Marion B. Coleman, Albany, N. Y.

Abstract. Epidemiological evidence has indicated cattle or dairy products to be the source of the incitant of almost all of the undulant fever in New York, the state from which the largest number of cases was reported during 1930. Goats have not been implicated and hogs could seldom be considered. Since certain authors have attributed the majority of infections in man, in the United States, to porcine strains of the abortus-melitensis group, to which cattle are also susceptible, a study of cultures from patients in New York State is of interest. The atmospheric requirements, absorptive properties, pathogenicity for guinea pigs, behavior in the presence of dyes, and the fermentation reactions with carbohydrates of the human strains were compared with those of representative cultures obtained from other laboratories and with those isolated from milk from

relation between the histological structure of the nodules in rheumatoid arthritis and in rheumatic fever. Last year we presented a more detailed study before the American Society of Clinical Investigation, further showing this relation. There is one point in which our studies do not correspond with those of Dr. Clawson. We were unable to obtain cultures of streptococci from the nodules. Of the organisms which we did obtain, one was a diphtheroid, and two were staphylococci. It was felt that these organisms probably represented contaminations from the skin.

"SENSITIVITY" TO SULPHYDRYL. Stanley P. Reimann, Philadelphia, Pa.

Abstract. Local areas of the skin of the arms of 450 humans were painted once with 1 per cent alcohol solution of thiocresol and controlled on the other arm with 1 per cent cresol solution. Of these, 18 individuals reacted by the production of an itching rash. The same phenomena occurred in a group of mice and rats.

Since the sulphydryl group is essential to cell division, it was thought that these individuals who are "sensitive" to this group are also "sensitive" to cell division phenomena. Adequate evidence on this point requires much more experimentation. In all probability, the reaction is allied to those by which different individuals respond to chemical groups, according to their nature and position in complex molecules.

KERNIKTERUS: JAUNDICE OF THE NUCLEAR MASSES OF THE BRAIN. H. M. Zimmerman and (by invitation) Herman Yannet, New Haven, Conn.

Abstract. Kernikterus is a condition of jaundice of various nuclear masses of the brain. The structures most commonly affected are the caudate, lenticulate, subthalamic and dentate nuclei, thalami, mammillary bodies, cornu ammonis formations, nuclei of cranial nerves, olives, parts of cerebellar cortex, and anterior and posterior horns of the spinal cord. It is most frequently, if not exclusively, associated with icterus gravis neonatorum, and its pathogenesis is through some injury to the nerve cells which are subsequently stained with bile pigments carried to them by the blood stream.

Detailed studies were made of the nervous systems of two infants who succumbed with severe neonatal jaundice, and who had kernikterus. There was, in addition, evidence of a suppurative omphalitis in each infant and of an acute hepatitis in one, from whom repeated blood cultures failed to yield any organisms. Postmortem blood cultures of the other yielded *B. coli*.

THE SUBDURAL SPACE, WITH SPECIAL REFERENCE TO SUBDURAL HEMORRHAGES. Timothy Leary, Boston, Mass.

Abstract. (A) *The Subdural Space:* The paradoxes which appear in connection with the subdural space reflect the differences in the origin of the pia arachnoid and the dura. The pia arachnoid, with cells derived from the neural crest (ectodermic), can limit extension of infection from the subarachnoid to the subdural space; can retain fluid in edema; can prevent invasion of meningiomata; does not organize and remove blood from the subdural space, perhaps because it is without capillaries. The dura (fibroblastic connective tissue) does not prevent extension of infection of its tissues to the subdural space; is invaded by meningiomata; organizes and removes blood from the subdural space.

was nineteen. In the father of this child the only symptoms were small hemorrhages per rectum and a positive agglutination test. The family was directed to observe other members of the family. A few days later the child developed mild meningeal symptoms and spinal puncture was done. The same findings as reported by Dr. Hansmann were present; the cells numbered 250, with 95 per cent lymphocytes; the organism was isolated from the spinal fluid.

We had a positive agglutination against a stock culture of *B. abortus* in a dilution of 1:640, with the organism isolated from the spinal fluid. We obtained a positive agglutination in a dilution of 1:1800.

The child recovered after an illness of two or three weeks, but subsequently developed a myelitis, so that the child at present is paralyzed below the mid-abdomen, with loss of control of the bladder and bowels, and total paralysis of the extremities.

STRUCTURE AND BACTERIOLOGY OF SUBCUTANEOUS NODULES IN CHRONIC ARTHRITIS. B. J. Clawson, Minneapolis, Minn.

Abstract. In 300 patients with chronic arthritis subcutaneous nodules were found in 90 (30 per cent). The nodules when removed often had a definite capsule, but in some cases this was poorly defined. When sectioned and examined grossly, multiple areas of necrosis were usually seen, surrounded by fibrous tissue. Necrotic and mucinous material could frequently be expressed from the center.

The nodules were found to be made up of multiple inflammatory areas. The centers of these commonly showed varying degrees of necrosis. Two types of structure were seen in the necrotic centers, a hyaline eosin-staining material and a fibrillar substance that stained with hematoxylin. Scattered in this necrotic material were varying numbers of polymorphonuclear leucocytes. Surrounding the necrotic centers there were many mononuclear and multinucleated cells (polyblasts) which varied in size and shape. Many of them resembled the epithelioid cells in a tuberculous lesion. These polyblasts generally, but not always, had a marked tendency to be arranged in a radial or palisade fashion. In this respect the arrangement was similar to that commonly found in the heart valve in acute rheumatic endocarditis. Polymorphonuclear leucocytes were scattered among the polyblasts, in many cases in small pockets or abscesses. These nodules simulate abscesses more closely than the acute rheumatic nodules which we have studied.

Streptococci were recovered in pure culture from 70.6 per cent of the nodules cultured.

The frequency of subcutaneous nodules in acute rheumatic fever and in chronic arthritis, the similarity of the gross and microscopic structure of the nodules in these two conditions, and the frequency with which streptococci can be cultured from the blood in acute rheumatic fever and from the blood and subcutaneous nodules in chronic arthritis strongly suggest that acute rheumatic fever and chronic arthritis have a common streptococcic etiology and that the two diseases are in all probability different manifestations of the same process.

Discussion

(Dr. M. H. Dawson, by invitation, New York City.) The paper is in large part confirmation of a study which Dr. Pappenheimer and I presented before this Society two years ago. In this study we were able to show a very close

MULTIPLE MALIGNANT TUMORS. Shields Warren, Boston, Mass.

Abstract. Forty multiple malignant tumors were found among 1078 autopsies on individuals dead of malignancy. Fifteen multiple malignant tumors were found in males, who died at an average age of 67 years, and twenty-five in females, who died at an average age of 58 years. The average known duration of the older of the two tumors was 3.2 years among males and 2.8 years among females.

On the basis of Massachusetts mortality statistics, adjusted for age and sex, 5.90 cases rather than 15 would be expected in males, and 3.85 rather than 25 in females, assuming that it is admissible to accept Wilson's method of applying cancer mortality rates as morbidity rates in calculating incidence of multiple malignancy, and that the second tumor is within one year of causing death.

On the basis of a group of 12,051 malignant disease autopsies collected from the literature, 54.2 cases would be expected from a similar calculation and 111 cases were actually found.

These findings would seem to indicate that multiple malignancies occur more frequently than chance would explain, and that some constitutional susceptibility to cancer must be assumed.

Discussion

(Dr. Howard T. Karsner, Cleveland.) I should like to ask Dr. Warren whether or not he applied strictly the Billroth criteria to the multiple large intestine tumors.

(Dr. Warren.) I should have stated that in the multiple large intestine tumors I had metastases in six cases where both tumors were of intestinal origin. In the ten instances of which I spoke, they were not all ten of the intestine, but one primary tumor occurred in the intestine and the other primary tumor in some other organ. Of course we recognize that polyposis as the basis for multiple malignancy may appear frequently, but without evidence of independent metastases I did not feel justified in including those cases.

(Dr. Alfred Plaut, New York City.) I should like to ask if the older statistics have applied the Billroth criteria to tumors which often do not metastasize, like carcinoma of the cervix; that does not seem probable, because it would defeat the purpose of the statistical work from the beginning. Secondly I should like to know if Dr. Warren has received the impression from certain cases that there may be something in the constitution of a human being which leads to the formation of many kinds of tumors. Probably many of us who do autopsies cannot help having such an impression. When a middle-aged woman has a carcinoma of the cervix, a myoma uteri, multiple tumors of the peritoneum, and in addition, a primary tumor of the kidney, one cannot help but think that there must be something in this body which leads to the formation of multiple primary tumors.

(Dr. David P. Seecof, Cleveland.) I should like to ask Dr. Warren how many cases there were in which the multiple tumors produced metastases, and whether there were any in this series conforming to Billroth's criteria.

(Dr. Warren, closing.) In regard to Dr. Plaut's first question, I have not my references here, so cannot answer it exactly, but in some of the series the definite statement is made that Billroth's criteria are applied. Those series run, as one would expect, very much less than the others. In other series the matter of

These paradoxes are best explained by a concept that the subdural space is *sui generis* among so-called serous spaces; that the skull with its lining dura forms an articulation, not with bone, but with soft parts. As in other articulations a true lining endothelium is absent or discontinuous (as shown by Mallory in 1920). The pia arachnoid is an essential part of the central nervous system and is covered by a continuous layer of ectodermic (?) cells. Its relation to the dura is largely one of contiguity, with continuity only at limited points.

(B) *Subdural Hemorrhages*: Primary hemorrhage into the subdural space arises almost exclusively from the pia arachnoid. Only when the dura is lacerated, or when tumors arising in or invading through the dura bleed, does primary hemorrhage of dural origin occur. The sources of primary subdural hemorrhage are so closely allied to the sources of subarachnoid hemorrhage that they must be studied together. Secondary hemorrhage of dural origin, due to rupture of new vessels organizing primary collections of blood, is common. Whether the primary bleeding be due to laceration of the pia arachnoid or to the rupture of arachnoid vessels, spontaneous (from veins usually), or due to minor traumatism, the blood in the subdural space is trapped. If the arachnoid is injured, repair, shutting off the membrane with adhesion to the dura, follows promptly. The dura surrounds the clot with a thin fibroblastic membrane separating the clot from the arachnoid, and a thicker layer lining the dura. From the dural side particularly, buttresses of young tissue enclose large vascular spaces, poorly supported, which are the probable source of secondary hemorrhages within the membranous sac. The organization is from one side of the clot and therefore slow and inefficient. As in extradural hemorrhages the only adequate treatment is operative removal of the clot. The increase in head injuries associated with automobile accidents makes recognition of this condition of primary importance, particularly in patients who recover from the immediate shock, but do not progress favorably.

THE HISTOGENESIS OF ATROPHIC CIRRHOSIS. Virgil H. Moon, Philadelphia, Pa.

Abstract. Atrophic cirrhosis is essentially a progressive chronic inflammatory process. Its development may best be studied in early and active cases. Such occur in children more frequently than in adults. Sections from active cases show progressive degeneration, necrosis and dissolution of liver cells associated with an inflammatory reaction, proliferative rather than exudative in character. Whole nodules are progressively destroyed and replaced by soft connective tissue. In adjacent areas proliferation of liver cells produces expanding nodules, which compress the recently formed fibrous tissue into bands. These nodules in turn are destroyed and the process is repeated. Cirrhosis occurring in adults is usually less active. Degeneration and destruction of liver cells followed by active tissue proliferation are less marked. Occasionally complete arrest of the cirrhotic process is apparent. Such cases show no degeneration and destruction of liver cells, or proliferating fibrous tissue. Pressure atrophy from contraction of fibrous tissue may be present. Such cases may not show clinical evidence of cirrhosis. Comparison indicates that the activity of the process frequently parallels the severity of the clinical symptoms. Streptococci were cultivated from the livers of six patients with active cirrhosis. They were demonstrated in stained section in each of these, and in other cases. In none of the cirrhoses in children was there history of alcoholism. In several cases there was recent history of scarlet fever. Infection is probably one important factor in cirrhosis.

particles of the cortex of the adrenals which have been implanted in the kidney during fetal life. We saw one of these illustrated here — a very convincing case from the interesting material of Dr. Crawford. My argument, which has not been used until now, is as follows. You all know the so-called suprarenal tumors:

- (1) The struma suprarenalis (nodules in the cortex)
- (2) The tumors of the suprarenal medulla
 - (a) Gangliocytoma and ganglioneuroma
 - (b) Chromaffin tumors (produce adrenalin)

It has been shown that the so-called accessory suprarenals sometimes contain medullary elements. If there is really an implantation of suprarenal elements in the kidney during fetal life, such implants may also contain *a priori*, at times, cortical and medullary substance. It follows, then, that such suprarenal elements in the kidney may form either hypernephroid tumors or gangliocytoma, ganglioneuroma, or chromaffin tumors, or even combined tumors of these types. Such tumors, as I have shown, exist in the kidney, and consist of a centrum of ganglioneuroma and of a cortex of a typical so-called hypernephroma. So here we have a combination of tumors of the same organ. Therefore you see there is surely the possibility that *pure* adrenal cortical tissue may also produce hypernephroid tumors in the kidney. It is very hard to tell how often that occurs, but there is no doubt that it does occur, and all I want to emphasize is that the general meaning of the hypernephroma of Grawitz is put on a real basis.

(Dr. William Boyd, Winnipeg, Canada.) It is very dangerous to draw conclusions from one case, because it is so easy to say that it is merely a coincidence, and yet if the coincidence is sufficiently striking, perhaps it has some degree of weight. I performed an autopsy some years ago in which I happened upon such a coincidence. The patient had died of a cerebral hemorrhage. It was a straightforward case, with no suggestion of renal disease or tumor, but at the autopsy I found a most striking adrenal rest in the left kidney. I do not remember ever seeing a really striking adrenal rest in the kidney in any other case. In this respect my experience is about the same as Dr. Crawford's. I was so struck by the rest in the left kidney that I turned to those in the autopsy room and said "Wouldn't it be funny if there was a hypernephroma on the other side?" — and there was! There was a hypernephroma of the right kidney. Possibly it was a coincidence, but it appears more probable that there was some causal relation between the two.

(Dr. Crawford, closing.) The number of cases here reported is too small from which to draw definite conclusions, but the data here presented added to the many previous reports are of distinct significance. I do not mean to imply that the adrenal tumors in the kidney do not occur, for this has been definitely proved in reports of typical cases by Professor Pick and others. The points that I wish to emphasize are that in this group the majority of the specimens present definite histological characteristics of carcinoma, and that true hypernephromas are much less frequent than carcinomas of the kidney, and that the adrenal rests occur so infrequently in the kidney as to be relatively unimportant in explaining the presence of the frequent malignant tumors of the kidney in adults. It does not seem reasonable to assume that these tumors are of adrenal tissue origin when they more closely resemble the cells of the kidney, and it would seem much more probable that they arise from preëxisting adenomas.

Billroth's criteria is discussed, and then the statement made that they are not utilized.

With regard to susceptibility to malignant tumors, I feel quite sure that we all have the impression that there is a definite susceptibility in certain persons to cancer, and I hope on the basis of a study such as this and others similar to it that we will be able to decide that question from a statistical standpoint, as well as from the standpoint of general impression.

With regard to Dr. Seecof's question, I will say in this series there were nine cases in which there were both primary tumors and metastases of those primary tumors present. In the remainder of the cases, in practically all of them, some one of the tumors had metastasized. There were eight cases in which neither tumor had metastasized.

THE CLASSIFICATION OF TUMORS OF THE KIDNEY WITH ESPECIAL REFERENCE
TO THE MALIGNANT TUMORS IN ADULTS. Baxter Lindsay Crawford, Philadelphia, Pa.

Abstract. This report is based on the study of 60 malignant tumors in adults, 4 malignant tumors in young children, and 29 benign tumors of the kidney which were discovered at autopsy. My chief interest has been in the study and classification of malignant tumors in adults. In this group of 60 cases, 59 have been classified as carcinomas and one as probably a hypernephroma. The 4 tumors in the children are either of the mixed tumor or embryonal type of carcinomas. Of the 29 benign tumors, 23 proved to be adenomas, 1 adrenal rest, and 5 fibromas or fibrolipomas. Everyone is familiar with the discussions which have appeared in the literature as to the origin of the malignant tumors of the kidney in adults since the theory was advanced by Grawitz in 1883 that these tumors arise from adrenal rests in the kidney. It is quite evident that many authors use the term "hypernephroma" to include all tumors of the kidney of a certain type, without reference to their origin. There would be much less confusion if the term "hypernephroma" were used to refer only to the ones which are considered to be of the adrenal tissue origin. It seems to be the consensus of opinion at the present time, of those who have made careful studies of large groups of these tumors, that the vast majority are true renal carcinomas and not adrenal tissue tumors. In the histological study of the majority of these tumors, true columnar epithelial cells forming indefinite acini and papillae may be demonstrated. Other points which support the view that the majority of these tumors are carcinomas instead of hypernephromas are the infrequency with which adrenal rests are found in the kidney, and the frequency with which adenomas are found in the kidney in various stages of development which may become malignant. In a series of over 2200 autopsies, I have found only one adrenal rest in the kidney, and in the same group, in something over 1 per cent of the cases, adenomas were found.

Discussion

(Dr. Ludwig Pick, by invitation, Berlin, Germany.) I am not surprised at the opposition of some people to the concept of hypernephroma, but it is a matter of fact, and not only a question of belief, that hypernephromas do exist, and I want to tell you, in addition to all other arguments, one point in support of this theory. As you all know, hypernephroma of the kidney is due to some

as well as the choroid, and also the glial cells in the peripheral and central nervous systems.

(Dr. S. Weintraub, New York City, by invitation.) Have you examined myelomas?

(Dr. Joseph McFarland, Philadelphia.) I recall when Dr. Laidlaw presented the photograph of the melanoma it consisted entirely of brownish cells, and he said he could not find any signs of malignant change. I wonder whether this reaction will enable us to decide if there is a malignant change in such tumors.

(Dr. Maurice N. Richter, New York City.) Dr. Laidlaw has been kind enough to perform the dopa reaction on sections of several cases of myeloid leukemia. We have found that the dopa reaction parallels the oxidase and peroxidase reactions rather closely. Unfortunately we have not had an opportunity of examining a case of myeloid leukemia in which the cells were sufficiently young for the reaction to be of much diagnostic importance. It is my impression that myeloid cells in the early stages, the so-called myeloblasts, do not give the oxidase or peroxidase reactions until they are sufficiently differentiated to show granules by other methods when suitably stained. In one case of acute myeloid leukemia which we have had recently, in which Auer bodies appeared in the circulating blood, Dr. Laidlaw demonstrated for us an Auer body with a positive dopa reaction in the tissue sections.

(Dr. Laidlaw, closing.) Concerning Paget's disease of the nipple, I have had no opportunity to try the dopa reaction. In ordinary epitheliomas a few dopa-positive melanoblasts are often seen among the basal epithelia. I do not believe that the presence of a few melanoblasts in an epithelioma has any significance, and certainly no relation to prognosis.

I have never examined the meninges. Bloch reports the melanin-containing cells as dopa-negative in the adult, sometimes dopa-positive in the embryo.

As for the pigment of the eye, I have had no experience. It is difficult to get fresh human eyes for a dopa reaction. Such eyes are usually dropped immediately into Müller's or Zenker's fluid in the operating room. The best work on the dopa reaction in the eye was done by Miescher, of Bloch's clinic, working with chicks and rabbits. He found the dopa reaction present only during a short period of embryonic life, both in the retina and in the choroid, while the pigment of the eye is being formed. After birth, the reaction is negative. If a malignant melanoma appears in the eye, the melanoblasts resume their embryonic activity and become dopa-positive again.

I have examined several pigmented adrenals. They were dopa-positive. The pigment was a lipoid and had nothing to do with melanin. In melanosis coli the pigment-bearing cells are dopa-negative. They are phagocytes and not melanoblasts.

I have done very little work on the blood and have formed no opinion of the value of the dopa reaction in identifying the early stages of blood cells. The subject awaits further study. In *Folia Hematologica*, of 1930, Bloch and Peck published a special technique for blood.

In regard to the nervous system, it has been reported that the ganglion cells are dopa-positive. I have been unable to confirm this. In my hands, all cells of the central and of the peripheral nervous system, both ganglion and glial cells, are dopa-negative.

The dopa reaction has no relation to malignancy, as you may see in the sec-

THE DOPA REACTION IN GENERAL PATHOLOGY. George F. Laidlaw (by invitation), New York City. ✓

Abstract. The dopa reaction is a specific stain for two kinds of cells, myelogenous leucocytes and melanoblasts. It is a valuable aid in the study of melanin production and the metastases of melanotic tumors. It identifies the actively functioning melanoblasts of the skin, of ectodermic mucous membranes, pigmented moles, melanoma and its metastases. When positive, the reaction distinguishes melanoblasts from phagocytes. A negative reaction has no meaning. The reacting substance disappears soon after death or after excision from the living body, and it is destroyed by most fixatives and preserving fluids. For melanoblasts, fresh tissue is required, or tissue that has been in 5 per cent formalin for only a few hours. Leucocytes may react after many days in 5 per cent formalin.

Discussion

(Dr. W. C. Hueper, Philadelphia.) I wonder if Dr. Laidlaw has examined the skin of the nipple. I think that the Dopa reaction should lend itself very well to settling several perplexing problems as to the origin of Paget's disease. If it is an intradermal cancer it should give a positive dopa reaction, and if it is a secondary reaction of a primary mammary gland cancer it should be negative.

(Dr. Victor Jacobsen, Albany.) This reaction should be of real service in determining the identity of cells containing brownish or brownish black pigment. We have heretofore not been able to say what a chromatoblast is and what a chromatophore is. The former cell is supposed to make pigment, and the latter only to carry it. This test apparently will settle the point. I should like to ask Dr. Laidlaw if he can throw any light by means of the dopa reaction on the actual identity of the pigmented cells which we find in the meninges, and which conceivably account for some of the malignant pigmented tumors of the meninges; also the pigmented cells of the chorioid and of the zona reticularis of the adrenal. We still have a problem ahead of us in settling the identity of intracellular pigment. Not all is gold that glitters, and probably all brownish black pigment is not melanin. We do not know what melanin is chemically. Usually it contains sulphur; it may have a little iron.

Further, how can we identify cells which do not contain pigment, but which are potentially melanin-producers? Can we take the skin of an albino animal and experimentally produce pigment by incubation for instance, which will make the cells sensitive to this test? In other words, should we consider R. Hertwig's old observation that melanin is a product of cell depression, and hence conceivably that it might be a product of practically any cell in the body? Hertwig's work was done with a unicellular organism, *Actinosphaerium eichornii*, but nevertheless I think it is of fundamental importance.

(Dr. Herbert S. Reichle, Cleveland.) I should like to ask if Dr. Laidlaw has used the dopa reaction for blood smears: I mean not only for the leucocytes in the tissues, but also for those in the circulating blood. If so, is it superior to the ordinary oxidase reaction? All of us know that the oxidase reaction often fails in the early forms of myeloblasts. Many of these do not give a positive oxidase reaction. I should like to know if the dopa reaction will give a positive reaction in such cases.

(Dr. George R. Callender, Washington.) I should like to extend Dr. Reichle's question and ask if Dr. Laidlaw has examined the pigment layer of the retina

Discussion

(Dr. W. C. Heuper, Philadelphia.) Can Dr. Strumia offer any explanation for the changes in the blood picture and the condition of the patient? I should like to call his attention to the work of Lindstroem with antileucocytic serum, who was able to produce in leukemic patients such conditions as appeared spontaneously in Dr. Strumia's patient, and by the injection of antileucocytic serum in animals he could even produce the death of the animal with the histological and hematological picture of typical agranulocytosis. I have worked with antileucocytic serum in tissue cultures, and I can say that such sera have a very marked and definite cytolethal effect on leukemic and normal human leucocytes. In Wells' Textbook of Chemical Pathology is a reference to the effect of X-ray therapy on leukemia, in which it is stated that X-ray therapy increases the antileucocytic titer in the blood, and the decrease in leucocytes in the irradiated blood may be due to an increase in the antileucocytic toxin in the blood. The last case which Dr. Strumia presented seems to be more of the type of mononucleosis in which the picture is similar to leukemia, with a marked increase in the mononucleated cells. The only difference between a typical leukemia and such monocytic conditions is the prognosis. The patient recovers if he has a mononucleosis, while the patient will not recover if he has a leukemia. I once described a case where a monocytosis was present with a total leucocyte count of 88,000 for some time, and the patient recovered.

(Dr. Strumia, closing.) It is rather hard to answer this question, because Dr. Heuper did not mention whether or not he means acute leukemia or chronic leukemia. I do not think the two diseases have anything in common except the name. That might create the impression that one is a phase of the other. That is unquestionably not so.

As far as the first of the cases is concerned, we did inject rabbit serum which had been inoculated with a suspension of white cells of the patient. The injections, however, three in all, were given ten days at least after the granulopenic phase had already begun. The change is probably brought about by a variation in the toxin or toxins which are likely the cause of the condition, together with a predisposing element, preëxisting in the patient.

In regard to the question of monocytic cells, there is no doubt that in the second case we are not dealing with monocytes. The cells were carefully studied in several hundred preparations. In cases of acute leukemia monocytes rarely occur, except in the so-called monocytic acute leukemia. In this particular case there were only occasional monocytes found, and in both cases most of the cells were of myelogenous origin, in the first one showing a fairly high percentage of oxidase-positive cells, and in the second somewhat lower. These cells were not hard to recognize in the films as being of myeloid origin. For the question of effect of X-ray in leukemia, I may refer Dr. Heuper to two works which I published some time ago.*

FOCAL ARTERIOLITIS. Alfred Plaut, New York City.

Abstract. In the course of several years a peculiar, circumscribed lesion of small arteries and arterioles has been found in twenty-four patients. Two of the

* Morphologic changes of the blood in myelogenous leukemia under radium treatment. *J. Lab. & Clin. Med.*, 1924, 10, No. 2.

On the generalized effect of radiations in myelogenous leukemia. *Am. J. M. Sc.*, 1929, 177, 676.

tions presented, where the cells of a benign mole react as strongly as the cells of a malignant melanoma. In saying that there were no signs of malignancy in the section, I meant the usual histological signs of mitosis and inflammatory reaction, not the dopa reaction.

CONCERNING THE NEURAL ORIGIN OF THE MELANOMAS. Nathan Chandler Foot, Cincinnati, Ohio.

Abstract. By means of lantern slides a series of sections are presented which very strongly confirm Masson's theories as to the neural origin of the melanomas. The photomicrographs were made from paraffin sections impregnated by the Rogers' silver method for the demonstration of neurofibrillae, as well as by methods adapted by the author, which are calculated to show the finer, connective tissue fibrillae.

Nerves and nerve terminals in normal tissue, Meissner corpuscles with their nervous apparatus, and so on, are shown; then the distribution of nerve filaments in benign melanomas is compared with these pictures and the close similarity between Masson's "lames foliacées" and the normal Meissner corpuscles is demonstrated. Besides these, more primitive fibrils and cellulofibrillar complexes are shown.

It is hoped, by means of this demonstration, that the theory of Masson is very firmly grounded and that there remains but little work to do before it may be proved conclusively. The cell nests resemble neither epidermoid nor connective tissue; they are almost invariably associated with nerve trunks; they contain numerous nerve fibers that tend to prove that the tumor cells are formed from the neural adnexa, as Masson has claimed.

ACUTE LEUKEMIA AND AGRANULOCYTOSIS. Max M. Strumia, Philadelphia, Pa.

Abstract. From the study of a large number of cases of acute leukemia, agranulocytosis and abnormal blood pictures occurring during the course of infections, especially streptococcic, the writer has previously suggested the hypothesis that there is a common causative mechanism in these various and apparently widely separated forms of blood disease.

Two cases are here presented. These must not be viewed as isolated, unusual cases, but rather as links illustrating the possible connections between acute leukemia and agranulocytosis:

Case 1 is that of a male, 21 years of age, who had, for a period of about a month during the course of an otherwise typical acute leukemia, a granulopenic phase with leukopenia as low as 400 cells per cmm.

After the granulopenic phase, the undifferentiated cells reappeared in the blood stream as rapidly as they had disappeared, the white blood count increasing in three weeks from 2100 to 430,000. The patient died with the blood showing again, as in the beginning of the disease, a typical picture of an acute leukemia.

Case 2 is that of a young girl, 9 years of age, who for six months had an agranulocytic blood picture. At this point, with only a slight increase of the total number of white blood cells (from an average of 2400 cells per cubic mm. to an average during the "leukemic" period of 3800) the patient exhibited for a period of a little over a month a typical picture of acute leukemia. This eventually disappeared and the patient had a slow recovery with a persistent moderate leukopenia and granulopenia, extending over a period of several years.

GLOMERULAR LESIONS ASSOCIATED WITH ENDOCARDITIS. E. T. Bell, Minneapolis, Minn.

Abstract. Two forms of glomerulitis — diffuse and embolic — are found in association with endocarditis.

Diffuse glomerulitis was found with the various types of endocarditis as follows: acute rheumatic 22.2 per cent; acute primary bacterial 28.6 per cent; subacute bacterial 64.8 per cent; secondary acute 33.3 per cent. It is characterized by an increase in the number and size of the endothelial cells and often by thickening of the capillary basement membrane. The extent of capillary obstruction is usually much less than in clinical acute glomerulonephritis, but in seven instances of subacute endocarditis glomerulitis had reached the clinical level. Diffuse glomerulitis bears some relation to the intensity and duration of septicæmia.

Embolic, or focal, glomerulitis was found in the different forms of endocarditis as follows: acute rheumatic 2.9 per cent; acute primary bacterial 7.1 per cent; subacute 52.8 per cent; secondary acute 5.8 per cent. In one instance there was no endocarditis.

Two distinct types of embolic lesions occur — the fresh hyaline and the fibrous.

The fresh hyaline lesion in its simplest form is a capillary thrombosis, and all the smaller lesions are readily recognized as such. The larger lesions are composed of many thrombosed capillaries which may be identified until the capillary walls have undergone necrosis. The hyaline lesion is not an infarct, but a thrombosis and necrosis of capillaries resulting from the lodgement of bacteria. The necrotic portion of the glomerulus disintegrates and disappears.

The fibrous lesion is a reaction characterized by a marked growth of the basement membranes of the capillaries. The thickened membranes obliterate the capillaries and give the glomerulus a fibrous structure. The fibers are formed entirely from basement membranes; there is no invasion by fibroblasts from without. The fibrous lesion, like the fresh hyaline, may involve one or more lobules, or the entire glomerulus. It develops independently of the fresh hyaline lesions.

In subacute bacterial endocarditis there were fifteen instances of severe renal insufficiency, of which five were due to embolic glomerulonephritis, seven to acute, and three to chronic glomerulonephritis.

The fresh hyaline embolic lesions develop earlier than the fibrous and may be found at any time during the course of the disease. The frequent absence of embolic lesions in typical clinical examples of subacute bacterial endocarditis has not been explained.

Diffuse glomerulitis is frequently found in association with embolic lesions.

Epithelial crescents frequently cause atrophy of the glomerular tufts by compression. Fibers form between the epithelial cells and convert the crescent into a dense fibrous structure. These fibers are of epithelial origin.

In the glomeruli, fibers which later give the staining reactions of collagen are formed from three distinct sources — intracapillary fibers from the endothelial cells, fibers formed from thickened capillary basement membranes, and fibers formed by the epithelial cells of the crescents.

lesions were found in the fallopian tube, the others in the vermiform appendix. The lesion consists of a subendothelial, hyaline deposit with necrosis of muscle coat and endothelium. The adventitia forms a granuloma consisting of spindle cells, mononuclears, irregularly round elements, and sometimes eosinophil and neutrophil granulocytes. The granuloma is well separated from the surrounding tissue. The lesion is distinctly focal. Generally several foci are found in the appendix.

There is no relation to any other disease. Only three of the patients were males, all the others females. This may be partly accidental. The age of the patients varies from 17 to 44. In fifty-nine autopsies, the appendix was examined, and in two instances focal arteriolitis was found, one patient being male. Focal arteriolitis is different from periarteritis nodosa. It is different also from the vascular lesion of typhus, and entirely different from the vascular lesions of rheumatic fever. The etiology of the disease, so far, is entirely unknown.

Discussion

(Dr. Virgil H. Moon, Philadelphia.) I am much interested in Dr. Plaut's presentation. I have not observed such lesions in human cases, but have seen them frequently in experimental animals. In a series of experiments, chronic foci of infection were produced with various organisms in order to study the prolonged effects of such infections. Focal arteriolar lesions, resembling those described, were found in a fairly high percentage of animals in which streptococci had been implanted. They were not produced by the streptococcus hemolyticus, but by streptococcus viridans, sometimes in association with lesions of the endocardium; at other times these arteriolar lesions were the only ones that indicated infection. They were found most frequently in the lungs, but were also found in cardiac muscle, intestinal walls, kidneys, and elsewhere.

(Dr. David P. Seecof, Cleveland.) Recently I have seen one case of this disease affecting every tissue in the body. That one case was found in a study of several hundred cases of arteriolar disease of the kidney. I saw this case within the last month, and it was in a human.

(Dr. Plaut.) In answer to Dr. Moon, I have to say that I have seen the lesion once — I found it accidentally in the skin of a young mouse. Nothing had been done to the mouse. I did not have any other organ of the mouse at my disposal.

As far as Dr. Seecof's remark is concerned, about arteriolar lesions of the kidney, was it a focal lesion?

(Dr. Seecof.) Yes. I have seen this lesion in the kidney often, but in this particular case every organ in the body was affected.

(Dr. Plaut, closing.) I have never seen this lesion in the kidney, and I wonder if I would recognize your lesion as identical with focal arteriolitis, or whether I should group it under arteriolar lesions we see in chronic kidney disease. Our lesion is distinctly focal. You might find a vessel involved for half a millimeter, and then it would be perfectly normal for a centimeter, and then there would be another area of involvement. There is no similarity between this lesion and arteriolo-necrosis. I have examined the spleen and pancreas of patients where we had reason to assume that the arteriolar lesion would be young, as in rapidly progressing chronic glomerulonephritis in children, but we never found anything similar to focal arteriolitis.

in recent years the attempt will be made to define the conditions under which these terms can be given greater precision. The cellular reactions of first infection and reinfection will be compared and their effect upon the invading microorganisms will be discussed. The uncertainty of available means for measurement of hypersusceptibility and resistance will be pointed out. The limitations of our knowledge concerning the relation of sensitization, allergy resistance and immunity to the clinical course and pathology of human tuberculosis will be cited.

Discussion

(Dr. E. M. Medlar, Mt. McGregor.) I should like to ask Dr. Opie how he distinguishes between an ulcer formed in the skin, an ulcer formed in the intestine, and a cavity formed in the lung in a tuberculous animal. I have yet to see any evidence of ulceration in which the principal cell participating in bringing about the reaming-out of tissue is not the polymorphonuclear leucocyte.

(Dr. Opie, closing.) It is not improbable that the polynuclear leucocyte has a part in cleaning off an ulcer, particularly when, as in the intestinal tract, mixed bacterial infection occurs. It seems to me unfortunate to use the word "abscess" to designate a tuberculous cavity. An abscess is characterized by accumulation of polynuclear leucocytes which set free a sufficient amount of proteolytic enzyme to bring about solution of dead tissue and injured cells, but with a tuberculous cavity there is accumulation of epithelioid cells, or necrosis caseation and disintegration of the caseous material. Polynuclear leucocytes may penetrate into this caseous material, and have some part in bringing about its softening, but the pathogenesis of an abscess and of a tuberculous lesion proceeding to softening are so essentially different that the term abscess should not be applied to a tuberculous cavity.

CHEMICAL FACTORS IN THE EXUDATION AND NECROSIS OF TUBERCULOSIS. Esmond R. Long, Chicago, Ill.

Abstract. It is well known that by the use of more or less purified products from the tubercle bacillus lesions can be produced in experimental animals which are closely analogous to those occurring in actual infection. These can be produced with such constancy that it is reasonable to suppose that these same substances are primarily responsible for the lesions actually observed in the disease. In recent years extensive research has been devoted to the purification of products from the tubercle bacilli and investigation of their effects in experimental animals.

The pathological effects to which injury has been directed in this study are exudation, in its widest sense, necrosis, proliferation and constitutional changes, as expressed by serum antibody reactions and related phenomena. In these effects the major fractions of the tubercle bacillus are concerned, either singly or, probably more commonly, in conjunction. In a general way the protein of the bacillus is responsible for the acute exudative phenomena of the disease, as manifested typically in the allergic reaction; the lipoids are largely concerned in the more chronic exudative manifestations and proliferative cell responses; the carbohydrates play a part in the serological antigen-antibody reactions.

The present paper deals with only a limited phase of this general problem — the more acute manifestations of exudation and necrosis. It is generally agreed

ACUTE DIFFUSE GLOMERULONEPHRITIS IN THE RABBIT. Kurt Semsroth (by invitation), Pittsburgh, Pa.

Abstract. Four to twenty-four hours after injection of a highly virulent pneumococcus Type 1 an acute reaction of all glomeruli of both kidneys was observed. Dominant features of the reaction were absence of early degenerative changes, enlargement of all glomeruli with capillary dilatation in the first stage, endothelial swelling and proliferation in the later stage. Since identical features set off the acute diffuse glomerulonephritis from any other kind of nephritis, the findings were interpreted as the analogue of the acute diffuse glomerulonephritis of man. Capillary dilatation was associated not with a hyperemia, but with a relative anemia of the glomeruli, while no "closing mechanism" at the vascular pole of the glomeruli was apparent. It was inferred that the primary phenomenon had been a widening of the glomerular capillary bed without a corresponding increase in blood-supply through the glomerular arteries. The observations led to the conclusion that the acute diffuse glomerulitis observed may be understood as due to the action upon the glomerular capillaries of metabolic products, which like urethane (Krogh) or histamine (Feldberg) have a dilator effect on capillaries, but none, or a constrictor effect, on arteries and arterioles.

UREA CLEARANCE FOLLOWING UNILATERAL NEPHRECTOMY. H. T. Karsner, R. A. Moore and (by invitation) R. F. Hanzal, Cleveland, O.

Abstract. The curve of urea concentration in the blood following intravenous administration of urea to unilaterally nephrectomized rabbits showed that after full recovery from operation the remaining kidney was capable of eliminating urea at the same rate as both kidneys.* In this series of experiments urea clearance of dogs was studied before, 1 month and 4 months after unilateral nephrectomy, by the method of Summerville, Hanzal and Goldblatt.† One month after unilateral nephrectomy, urea clearance under natural conditions was found to be unchanged, but if an excess of urea were administered, the urea clearance was slightly depressed as compared with the controls. At 4 months after unilateral nephrectomy, or later, urea clearance under natural conditions was not significantly changed, but with an excess of urea in the blood it was definitely increased. After full recovery from operation, and presumable increase in size of the remaining kidney, that organ was found to be capable of eliminating urea at a rate in excess of that of both kidneys before operation, a manifestation of genuine hypertrophy.

THE CELLULAR REACTIONS OF TUBERCULOSIS AND THEIR RELATION TO IMMUNITY AND SENSITIZATION. Eugene L. Opie, Philadelphia, Pa.

Abstract. The relation of tuberculosis to other forms of bacterial infection will be discussed. There is evidently wide difference of opinion concerning the significance of terms such as inflammation, exudation, and so on, as applied to tuberculosis, and even more uncertainty concerning the significance of sensitization, immunity, "allergy," and so on. With the aid of observations made

* Karsner, H. T., Straus, R., Moore, R. A., and Hanzal, R. F. Urea tolerance after unilateral nephrectomy in rabbits. *J. Exper. Med.*, 1932, 55, 27.

† Summerville, W. W., Hanzal, R. F., and Goldblatt, H. Urea clearance in normal dogs. (In Press.) *Am. J. Physiol.*

fibers, and an argyrophil tendency in the glue-like exudate of the peritoneal cavity, suggest that products of degenerated collagen may play an important part in the composition of exudates in tuberculosis.

Discussion

(Dr. Camille Kereszturi, New York City.) I should like to ask Dr. Long to interpret an observation of ours. We watched nine children who, after parenteral BCG vaccination, had a negative tuberculin reaction to 10 mg. OT. A few days after this test the interne made a mistake in the dilution of the tuberculin and gave 100 mg. instead of 10. To this dose all children developed a positive reaction, *i. e.*, 8 to 10 mm. erythema and infiltration with a small blister. I want to ask Dr. Long how he interprets this. Were these children slightly sensitive to tuberculin giving a negative reaction to 10 mg. of OT, or did the highly concentrated tuberculin give a mechanical reaction, a sort of foreign body reaction, or did only some component of the tuberculin produce the reaction?

(Dr. Long.) May I ask what kind of tuberculin you used for the test? That might make a difference.

(Dr. Kereszturi.) New York Health Department OT.

(Dr. Long, closing.) I have always felt that in tuberculosis there are varying degrees of sensitization that may be brought to light by varying the amount of tuberculin which is injected to produce a reaction. It would be my explanation that small amounts of tuberculin would not bring out the reaction, when the sensitization is very low, while larger amounts might induce a positive test.

I should like to say at this point that it has seemed to me that the more highly purified materials we use for the tuberculin test, the more sure we are of the results. In old tuberculin there are a great many substances of more or less unknown nature, including proteins, beef extract and peptone, as well as a large amount of glycerol and inorganic salts, and while the reactions with this material may be excellent, and the material may often behave as well in the testing treatment as other forms of tuberculin, it seems to me that when we have materials which do produce at least equally good reactions which are single pure substances, we are much more sure of the interpretation of our results than with material which contains so many unknown substances. Whether these unknown substances may be responsible for the reaction you speak of, I do not know, but I should have to consider it as a possibility.

THE SENSITIZATION OF GUINEA PIGS WITH TUBERCULIN AND THE PRODUCTION OF ANAPHYLAXIS AND ALLERGY TO THE TUBERCULO-PROTEIN. Herbert S. Reichle (by invitation) and Harry Goldblatt, Cleveland, Ohio.

Abstract. Normal guinea pigs were injected intracutaneously with 1 to 10 old tuberculin and various adjuvant substances, such as eye fluid of normal guinea pigs and horse serum. Upon retesting these animals with old tuberculin 3 to 8 days after the sensitizing injection, the animals responded in a fashion typical of bacterial allergy. Fifty-five of 102 animals used in these experiments have shown this phenomenon.

The skin reactions were of the delayed type, and although vesiculation and ulceration were never seen, they were otherwise analogous to those observed in tuberculous animals. The same reaction was obtained with Seibert's pure tuberculo-protein. At an early stage of an experiment the animals were not

that marked exudation in tuberculosis is a reaction of reinfection, although it is admitted that enormous infecting doses, larger than those operating in actual infection, can be a primary cause of exudation. It is now well known that, in addition to actual infection, inoculation with dead bacilli will achieve a sensitiveness similar to that of infection, and it has recently been shown (Seibert) that the purified protein also will sensitize so that exudative reactions will follow its reintroduction. There is rather general agreement that a protein or protein derivative of the bacillus is the substance responsible for the exudation itself.

There is no general agreement on the etiology of the necrosis of tuberculosis, probably because of the multiplicity of factors concerned. A distinction must be drawn between acute necrosis and the more slowly developing process of caseation, but the two are frequently associated, and the first may pass into the second. According to the old view (Virchow) arising with the development of the cellular pathology, the stages in the necrosis of tuberculosis were epithelioid cell proliferation, ischemia, necrosis. Weigert considered it a form of coagulation necrosis. To-day stress is laid by many (Krause, Rich, Huebschmann, Schleussing) on the rôle of hypersensitiveness in the development of necrosis. Others (Sabin, Medlar) have stressed the relation of necrosis to the life history of certain cells. Sabin and her coworkers in particular have focussed attention on the stimulation of the monocyte by the phosphatide of the bacillus, the maturation of this cell into an epithelioid cell and its final disintegration with continued phosphatide stimulation. It is important to note that in the necrotic tissue of a tuberculous lesion several elements may be present, including the original tissue, the inflammatory exudate or proliferate, and a fibrillar ground substance, which is commonly overlooked because not brought out by the usual stains.

Acute exudation and necrosis can be readily produced in tuberculous or otherwise sensitized animals by small amounts of the purified protein obtained from the tubercle bacillus or from the culture medium on which it has grown. Quantities of this protein as small as 0.1 mg. can kill a sensitive guinea pig in eighteen hours on intraperitoneal injection. In such animals 5 to 15 cc. of exudate is poured out in the peritoneal cavity. This fluid ranges from clear to cellular and bloody, according to the intensity of the reaction. Even the clearest samples have a tendency to gel, although fibrin cannot be demonstrated in the early reactions. The fluid is alkaline (pH 7.6 or more alkaline) and rich in protein. Non-protein nitrogen is relatively high. When fixed in Zenker's fluid it fails to take fibrin stains, but stains feebly with the Mallory stain and with silver.

Intense allergic reactions can be produced with the purified protein in a variety of tissues besides the serous membranes, including the skin, testis, lung, kidney and cornea. The reaction in the latter is especially instructive. Small amounts of the protein in the cornea of a tuberculous guinea pig cause an intense exudation of polymorphonuclear leucocytes and at the same time necrosis of the connective tissue, preceded by marked swelling and loss of staining capacity of the collagenic fibers. At the same time argyrophil fibers make their appearance. These seem in large part to be due to a simple mechanical separation of the collagenic fibers by the exudate, a result in agreement with the views of Mallory and Parker on the formation of reticulin, but may be in part a new formation secondary to the degeneration and solution of the collagen in the inflammatory exudate. The presence of faintly argyrophil fibers in the anterior chamber of the eye after an intense corneal reaction with destruction of the collagenic

be obtained with any of the constituents isolated from OT similar to those produced by the highly purified tuberculin protein. Therefore we purified OT by the ultrafiltration method and obtained a colloidal solution containing protein and cabohydrate. This, after seven to eight intracutaneous injections into a normal rabbit, gave a marked Arthus reaction, with considerable induration and hyperemia and only a little necrosis. Therefore, in OT there is still a part of the protein left intact antigenically, in spite of the hour or so of heating in its manufacture.

However, when this colloidal solution was precipitated with trichloroacetic acid, a protein fraction was obtained which had about 14 per cent nitrogen and many of the characteristics of protein. Nevertheless this fraction was a poor antigen, giving scarcely any reaction following seven to eight injections into the skin of normal animals. On the other hand, it did give a reaction in the skin of tuberculous animals.

We are of the opinion, then, from this work, that it is possible to modify the tuberculin protein to some extent, as is true in the case of OT, and still get an antigenic reaction. Further chemical treatment, such as precipitation with trichloroacetic acid, decreases its antigenic capacity so that it will elicit a response only in a highly sensitive animal, such as a tuberculous animal. Such a product may therefore be best for diagnostic purposes. We are very much interested in studying the chemical differences in this product and in the purified tuberculin protein.

(Dr. Reichle, closing.) In reply to the first question, we did try to inject animals intravascularly with OT. There are two objections. In the first place, it has been shown that OT, possibly because of the phenol contained in it, will in large enough doses kill normal guinea pigs. The second objection is one to which Dr. Karsner has called attention, the anaphylactoid reaction, or whatever the phenomenon may be that is connected with the injection of colloids into the vascular systems of animals. We did obtain shock in the animals. One showed typical emphysema, but we do not feel that this method is as favorable as the objective demonstration by means of the Dale method.

I do not quite understand what Dr. Aronson wishes. If he thinks that individuals injected with tuberculin may later show an anaphylactic response to tuberculin, I have no doubt that he is correct. Whether tuberculin contains protein or not, I am not in a position to say, and I must admit when I started this work we were of the opinion that it did not. Apparently Dr. Seibert's work shows that there is some, enough to produce a biological reaction. Some may prefer to call OT a haptene, although I question the validity of its use in this case.

The rôle of glycerin broth and the possibility of its giving a tuberculin reaction is such an involved subject that I think we cannot discuss it in this brief time. Within the next few years we will perhaps discover that there are special lots of glycerin broth which have some chemical and immunological relation to tuberculin. There have been many reports from Europe and America. I am not at all sure that glycerin broth cannot possibly give reactions in infected animals which are like those given by OT.

The work of Dr. Seibert I hardly need to discuss. It is a very interesting point. I wish to say that we have been in correspondence with Dr. Seibert, and that she has been very kind in giving us information and material.

sensitive to glycerin-bouillon, but after repeated injections with old tuberculin they developed sensitivity to the glycerin broth. In some of the animals the Long testicular test for allergy to tuberculin was positive; in others a strong anaphylactic sensitivity to tuberculin was demonstrated by means of the Dale test.

It is probable that an adjuvant substance is not a necessary factor and that the essential element in all previous reports of successful artificial sensitization to tuberculin has been the tuberculin itself. Some of the reasons why others have not been able to substantiate these claims are probably the failure to recognize the incubation period, the use of animals below 500 gm. and the lack at that time of an objective measure of allergy, such as the Long testicular test.

Discussion

(Dr. Max B. Lurie, Philadelphia.) Did these animals die in typical anaphylactic shock when properly tested by the antigen?

(Dr. Joseph D. Aronson, Philadelphia.) There are a number of points with which I disagree with the speaker. Old tuberculin is not antigenic and does not sensitize to itself. The late Dr. Paul A. Lewis showed that sensitization due to large amounts of tuberculin was brought about not by tuberculin, but by constituents of the broth. In so far as tuberculo-protein is concerned, its antigenic properties are different from that of old tuberculin. In his classical studies on tuberculo-protein Baldwin showed that tuberculo-protein acts as does any native antigen, and that anaphylactic shock may be produced in the non-tuberculous sensitized guinea pigs. Zinsser showed that the isolated uterus of guinea pigs sensitized with tuberculo-protein contracts when brought in contact with the antigen. We have found, as I have no doubt have other investigators, that tuberculo-protein injected into normal guinea pigs sensitizes them so that upon reinjection they die from anaphylactic shock; the isolated uterus of such sensitized guinea pigs contracts when brought into contact with the antigen. Rabbits injected with tuberculo-protein develop an Arthus phenomenon and have demonstrable antibodies in the circulating blood. In conjunction with Dr. Nicholas of the Children's Hospital we have been testing children simultaneously with old tuberculin and with tuberculo-protein. We have found that in so far as the sensitivity of the two substances is concerned, both give about the same per cent of positive reactions. However, when these children were retested three months later with tuberculin and tuberculo-protein it was found that about 40 per cent gave an Arthus type of reaction, and only about 4 per cent reacted to the old tuberculin. The Arthus type of reaction was characterized by its more prompt appearance, reaching its maximum in twenty-four hours, marked edema in a number of instances, and a subsidence of the edema after forty-eight hours. Many of the children complained of pain at the site of injection, a symptom absent when first injected. In view of our findings, I feel that tuberculo-protein as at present prepared cannot replace old tuberculin because of the danger of sensitization.

(Dr. F. B. Seibert, Chicago.) In this connection I should like to mention a few experiments which we have done this year on the purification of OT. The results which we obtained, but which I cannot describe in detail to-day, make me believe that the reactions produced by Dr. Reichle are due to the protein portion of OT. We were interested in finding whether antigenic reactions could

and subcutaneous inoculation, reaches its maximum response between 4 and 8 weeks and may persist as long as 240 days after inoculation. The most durable and intense cutaneous allergy was produced by the intradermal inoculation of BCG and the most rapid and least stable allergy was observed following the intraperitoneal inoculation of BCG. The oral administration of 10 to 35 mg. of BCG in newly born guinea pigs yielded irregular cutaneous allergy.

Guinea pigs inoculated with BCG and subsequently, during the anergic phase, inoculated subcutaneously with a minimal infecting dose of 200 living "H₃₇" virulent tubercle bacilli, showed an increased resistance to the tuberculous infection and survived twice as long as the unprotected controls. The greatest resistance obtained in the group inoculated intradermally with BCG and the least resistance was shown in the group inoculated intraperitoneally. Thus cutaneous allergy followed closely the degree of resistance of a virulent tuberculous infection. Animals vaccinated by the oral route showed the least resistance to infection.

These investigations uphold Calmette's original contention that the BCG culture is incapable of producing progressive tuberculosis in the animal body and that it may be used without risk as a vaccine. In my series of experiments the average survival time of BCG protected guinea pigs was 50 weeks and that of the control was 27 weeks, a difference of statistical significance.

Discussion

(Dr. William H. Park, New York City.) I have been very much interested in this paper because I have been planning for some time to do what Dr. Birkhaug has done, but have not had the facilities where the animals could live in a proper way. My interest was to see whether the BCG children lose their tuberculin test, whether they are equally or less resistant, and how long that resistance lasts. I do not know whether Dr. Birkhaug can tell us how long that resistance lasts when a tuberculin injection has become negative. I think we have also come to the same conclusion that he has, that the oral vaccination was not nearly as effective as the intradermal or subcutaneous methods, and that the intradermal was possibly the best.

(Dr. Joseph Aronson, Philadelphia.) I wish to state that our results agree with those of Dr. Birkhaug. We have found that guinea pigs vaccinated either subcutaneously or intracutaneously with the BCG vaccine and subsequently infected with a virulent culture of the tubercle bacillus survive for a longer time than do control unvaccinated guinea pigs. Guinea pigs vaccinated subcutaneously with the BCG vaccine and subsequently infected intratracheally with a virulent culture of the tubercle bacillus show definite fibrosis of the hilum lymph nodes, an occasional tubercle in the lung and little or no involvement of the spleen, whereas unvaccinated guinea pigs or guinea pigs vaccinated with heat-killed cultures of the tubercle bacillus show caseous hilum lymph nodes with extensive involvement of the spleen. We have found that the R 1 strain gives results as good as the BCG vaccine.

(Dr. S. A. Petroff, Saranac Lake.) The paper presented by Dr. Birkhaug is very interesting. It supports the observations made by the majority of investigators. My interest in this organism dates back to 1925, when I began my experiment. Contrary to Calmette's claim, I observed progressive tuberculosis in guinea pigs. I was one of the first to call attention to the fact that the organism (BCG) at times, when cultivated in a certain environment, may become viru-

A STUDY OF THE PATHOGENICITY OF THE BACILLUS OF CALMETTE-GUÉRIN (BCG). William H. Feldman, Rochester, Minn.

Abstract. Using a strain of BCG obtained from Calmette of the Pasteur Institute a deliberate attempt was made to increase its pathogenicity by subculturing the organism on a glycerinated egg medium. Transfers were made every thirty days. From each succeeding subculture four guinea pigs were injected two intracerebrally, one subcutaneously, and one intraperitoneally. The report deals with data obtained after the organism had been subcultured on the glycerinated egg media for fifteen generations.

Of a total of fifty-eight guinea pigs inoculated, lesions histologically indistinguishable from those of genuine tuberculosis occurred in the tissues of ten and cultures of acid-fast bacilli were obtained from each. While the majority of the lesions occurred in animals that had been injected intracerebrally, one animal injected intraperitoneally and another injected subcutaneously developed lesions of a tuberculous nature and died. So far attempts to promote a succession of tuberculous lesions by the re-inoculation into guinea pigs of infective material from lesions have failed.

Conclusions: 1. The particular strain of BCG studied is not devoid of pathogenicity for guinea pigs and the assertion that the organism is innocuous cannot be accepted without reservations.

2. Subculturing the organism on glycerinated egg medium at monthly intervals for a period of fifteen generations did not markedly enhance its virulence.

3. The state of an animal's resistance or susceptibility is perhaps of prime significance in determining whether a given individual may or may not develop tuberculous lesions following an exposure to BCG.

PROTECTION AGAINST TUBERCULOSIS WITH BCG VACCINE IN GUINEA PIGS.
Konrad E. Birkhaug, Rochester, N. Y.

Abstract. This communication deals with a 2 year investigation of the virulence of Bacillus Calmette-Guérin (BCG), the allergic response and increased resistance against virulent tuberculous infection following vaccination of guinea pigs with the living BCG culture. My strain (BCG-Park No. 10) was subcultured according to Calmette's directions on bile-glycerine potato, glycerine potato, and Sauton's synthetic medium, as well as on Dorset's glycerine egg medium, and Petroff's gentian violet egg medium. The virulence of the whole BCG culture, as well as dissociated "S" colonies of this culture, was tested on normal guinea pigs with proper controls, by inoculating from 1 to 35 mg. (dry weight) of the culture intradermally, intraperitoneally, subcutaneously and orally. Enhancement of virulence was also attempted by Dreyer's method of deep bouillon cultures. The results of these experiments have shown that BCG retains a high degree of stability, both in cultures and in the animal body, and that my strain is completely avirulent for guinea pigs. This culture is capable of producing tuberculous lesions of purely localized nature without killing the animal for a period of 2 years after inoculation. The lesions heal spontaneously by cicatrization within 8 to 10 weeks after ulceration commences, leaving only a superficial white scar with slightly enlarged adjacent lymph nodes. The BCG strain remains viable in the caseo-purulent contents of superficial abscesses as long as 98 days after inoculation and remains avirulent when subsequently inoculated into normal guinea pigs. Cutaneous tuberculin allergy develops regularly within 2 to 4 weeks following the intradermal, intraperitoneal

(Dr. Max B. Lurie, Philadelphia.) I wish to call attention to some work done in Germany recently by Tiedeman in Kirchner's laboratory, which showed that one cannot necessarily draw any conclusions from the behavior of the guinea pig as to the behavior of the human being toward BCG and other strains. For example, in the strains isolated from the Lübeck disaster, it has been found that while these strains caused the most progressive and most fulminating type of tuberculosis amongst infants, this same strain when injected into guinea pigs caused a very slowly progressive disease chiefly limited to the lymphatic system. This work was confirmed in several laboratories. The conclusion seems to be well established on the basis of other facts that the tubercle bacillus in general may be much more virulent for the human being than for the guinea pig and that the BCG also may be much more virulent for the human being than for the guinea pig.

(Dr. Park.) We have now been carrying these children for five years, and have not the slightest evidence that it does them any harm, and we have evidence that the resistance given is not so great. When they developed whooping cough, two died of the human type tuberculosis. The abscesses always heal up when they develop them.

(Dr. Birkhaug, closing.) I believe there is one cardinal difference between my experiments of protection by means of the BCG vaccine and those of other workers, in that too much haste was exercised in inoculating the virulent tubercle bacilli into the vaccinated guinea pigs before any proof was available that cutaneous allergy was established. I purposely planned to defer the superimposed virulent infection for as long as five months after the BCG vaccination, until I had tangible proof that allergy was established and most of the animals had passed into the anergic state. In this connection there seems to be little doubt now that allergy is a definite expression of immunity and that in many instances cutaneous allergy in the guinea pig is not established before eight to ten weeks following the BCG vaccination. I like to stress this significant difference between my experiments and those of other workers.

During my six months' visit last year to European centers of human vaccination with BCG, I learned something about proper dosage. The most unique results were obtained by Dr. Wallgren, at Gothenburgh, Sweden. His chief concern was to correlate dosage with production of cutaneous allergy and production of cold abscesses. His dosages were graduated from 0.2 to 0.5 mg. The latter dosage injected subcutaneously produced the least cold abscesses and the highest allergy. About 76 per cent of infants given one dose of 0.05 mg. became tuberculin hypersensitive from 4 to 9 weeks after vaccination. Another significant feature of Dr. Wallgren's series was his insistence on strict isolation of the vaccinated infant from open and virulent tuberculous infection until cutaneous allergy was established. This ideal arrangement necessarily entails financial difficulties, both for the experimental station and the parents, coupled with the disinclination of many mothers to be parted from their infants during the necessary period of isolation.

In regard to the question about the possible enhancement of virulence of the BCG organisms enough has been said. Although no living virus is absolutely fixed in virulence or avirulence, there is but most meager evidence that BCG is capable of producing progressive tuberculosis in animals or man. An overwhelming majority of workers have adequately confirmed Calmette's thesis in this respect.

The Lübeck tragedy was unique in medical annals. Again, it should be re-

lent for guinea pigs. I attempted to connect the instability of this organism with the dissociation phenomenon. From the original culture I dissociated two extreme types of organisms, which differed in topography of the colonies, virulence and other biological reactions. My investigations were not corroborated until recently. We must remember that the tubercle bacillus grows very slowly and takes a long time to be modified. In 1928 I brought with me from the Pasteur Institute a culture of BCG (359). A suspension prepared as described by me was inoculated in ninety plates, and in only one of the plates three typical "S" colonies developed corresponding very closely to one which I had previously reported. From this it is evident that a great deal of patience is necessary in order to succeed in dissociating it.

Seiffert of Freiburg recently stated that at least two years are necessary to dissociate the BCG cultures. It is not surprising, then, that many in the past have failed to corroborate my claims.

Concerning immunity in guinea pigs, established after the vaccination with BCG, we failed to observe such immunity as Dr. Birkhaug reports. There is a degree of protection in vaccinated animals, but it is no greater than that established when heat-killed organisms are used. I know of no experiments, other than this one reported by him, where the protection was so great. Even the most ardent supporter of vaccination has failed to establish such immunity in guinea pigs. Professor Calmette has stated repeatedly that small laboratory animals are not suitable for testing the immunity established by this organism.

I hope that the results reported here this afternoon can be repeated and confirmed by other workers. I still believe that this method of vaccination should be a problem of the laboratory where all the evidence can be properly weighed. If the vaccine is found to be innocuous and efficient, only then should it be released for use on human beings.

(Dr. E. M. Medlar, Mt. McGregor.) In regard to BCG becoming virulent, I wish to cite one instance: about two and a half years ago, we inoculated a series of guinea pigs with BCG mixed with SiO_2 . About six months ago we killed the remaining animals. None of them died of tuberculosis. There was one which had a slight enlargement of a lymph node on the same side in which the BCG had been inoculated. By inoculating this into another animal, we got progressive tuberculosis. The animal died within two months.

(Dr. Camille Kereszturi, New York City.) It is interesting that Dr. Birkhaug used egg medium. There is evidence in the literature that egg medium may increase the virulence and growth of BCG. However, in Dr. Birkhaug's experience, this did not happen, and in Dr. Park's it did not either. Dr. Park feels that the egg medium probably increases the growth of BCG and not the virulence.

A thing I should like to ask Dr. Birkhaug to do is to inject his animals in the stage in which they are positive to tuberculin, and inject others in the stage when they are negative. It is important for us to know whether we should consider children immune or relatively immune after they have lost their positive tuberculin test.

The next thing I should like to ask Dr. Birkhaug is whether he would be kind enough to try different BCG doses, because we are using much smaller amounts in children. Our highest dose is 0.3 mg. by injection. If he gives to a guinea pig 20 mg. and has good results, this might mean that we should increase our dose for children. Our chief difficulty is the determination of the ideal dose for children. Animal experiments like his might help us somewhat in estimating the desirable dosage for humans.

karyocytes is admitted. The evidences for and against the distinct identities of the typical Langhans' giant cells and the foreign body giant cells is weighed and the absence of dependable proofs of their separate natures emphasized. The conclusion is reached that they are probably different manifestations of one and the same cell.

Discussion

(Dr. C. A. Doan, by invitation, Columbus, O.) I do not think we differ very much with either Dr. Medlar or Dr. Haythorn in the interpretation of giant cells. We do find polymorphonuclears within the epithelioid or so-called rosette type more rarely than in the so-called foreign body type. There is less cytological differentiation in considering fixed tissues than in the supravital methods. There seems to be no question in either type of material that both fusion and mitosis occur in the development of multinucleated cells. The opportunity to observe the presence of a rosette in cells when they are stained with neutral red sharply differentiates them from those without rosette and with nuclei that are scattered diffusely through the cell, the so-called foreign body type; such criteria in a fixed preparation would not be so apparent. With reference to epithelioid giant cells, I think supravital studies show that the nuclei are restrained to the periphery because of this rosette body in the center of the cells. In the very earliest tubercle made up of epithelioid cells, which are proliferating rapidly, it has been our experience in supravital studies that the so-called foreign body type of giant cell is exceedingly rare. In the early stages before there has been necrosis with attendant foreign body reaction, only the smaller multinucleated cells with central rosette are present. There is no question, however, that later on we have a mixture of both types of cells, or the two morphological expressions of one type, whichever interpretation you care to make.

THE EFFECT OF VIRULENCE OF TUBERCLE BACILLI ON THE HISTOPATHOLOGY OF TUBERCULOUS LESIONS IN NORMAL ANIMALS. E. M. Medlar and (by invitation) K. T. Sasano, Mt. McGregor, N. Y.

Abstract. This study was made with two strains of tubercle bacilli, one of bovine origin and one of avian origin. In each instance we had cultures of high and of low pathogenicity of the same strain. The animals were all inoculated intravenously. In the case of the cultures of high virulence all of the animals died within two months. In the animals inoculated with bacilli of low pathogenicity none died even if allowed to live as long as two years.

The essential differences in the pathology are as follows: The chief lesions produced by the bovine cultures of high virulence were abscesses which ruptured and caused a "spreading" of the disease and by the virulent avian bacilli of extensive mononuclear infiltration with necrosis of these cells and subsequent neutrophilic infiltration. The chief lesions produced by the bacilli of low pathogenicity were mononuclear tubercles, often with giant cells present; collections of giant cells, often with pigment; large collections of lymphocytes, and scars with little lymphocytic infiltration.

From these studies it is evident that the typical text-book description of "tubercle" represents a retrogressive or healing phase of tuberculosis. The same is true of lesions where scar tissue and lymphocytic infiltration predominate. Such lesions represent the pathology in normal animals infected with bacilli of low pathogenicity or the lesions of successful resistance in animals or human beings infected with bacilli of high virulence. Bacilli are absent or extremely rare in such lesions.

emphasized that the impartial Superior Court at Lübeck clearly vindicated the harmlessness of the BCG vaccine. I believe one should be satisfied with this decision.

NEW STUDIES ON THE FILTRABILITY OF PURE CULTURES OF THE TUBERCLE GROUP OF MICROÖRGANISMS. Ralph R. Mellon and (by invitation) L. W. Fisher, Pittsburgh, Pa.

Abstract. The experiments to be reported have been designed to overcome the last remaining obstacles to the acceptance of the view that the acid-fast group of bacteria possess a filtrable phase in their life cycle. The matter is thus stated because we have employed chiefly a timothy bacillus rather than the tubercle bacillus, although even with the latter, complete parallelism exists as far as we have gone. The organism in question grows as the tubercle bacillus, is acid-fast, and is indistinguishable morphologically from this organism.

Summed up, the links in the chain of evidence are as follows: First, demonstration that the acid-fast gonidia of a granular sputum pass the filter; second, although greatly attenuated they will cause typical tuberculosis from which the virulent organism may be recovered; third, that the timothy bacillus in pure culture forms gonidia which will germinate into the original strain or one or more intermediate diphtheroid strains; fourth, that identical diphtheroid strains dissociate spontaneously without filtration; fifth, that diphtheroids with abundant acid-fast granules dissociate from pure cultures of both bovine and avian tubercle bacilli.

Discussion

(Dr. N. W. Popoff, Rochester, N. Y.) In the experimental studies on filtrability of tuberculous microörganisms the technique used is of essential importance, and for this reason the results reported here should be interpreted with great caution. In such experiments the main thing is the type of filter used. In his monograph on ultravirus Hauduroy says that filtration through porcelain bougie, infusoria earth, caolin, and so on, must not be used any more in scientific research. The studies on tuberculous ultravirus published recently by Sanarelli and Alessandrini are of great significance. Their excellent methods discarding entirely the use of Chamberlain and Berkefeld filters ought to be considered as a fundamental prerequisite in research studies on bacterial filtrability.

(Dr. Fisher, closing.) The filters used were the Berkefeld N's and we are not assuming that they filter the normal tuberculosis organism, but rather the gonidial granules or their subdivisions. Under the conditions of the experiments we are unable to filter the tubercle bacillus itself. We do get these non-acid-fast forms after a period of incubation. We are at the present time attempting measurement of these granules by indirect methods. Our contention then is for a filtrable phase in the life history of the tubercle bacillus, biologically distinct from its "normal" acid-fast phase.

EVIDENCES OF THE NON-SPECIFIC NATURE OF THE GIANT CELL OF TUBERCULOSIS. Samuel R. Haythorn, Pittsburgh, Pa.

Abstract. Four kinds of so-called multinucleated giant cells which have been described in tuberculosis are discussed. The common occurrence of structures morphologically resembling giant cells, but in reality only caseous foci with marginal phagocytes is demonstrated. The occasional participation of mega-

but occasionally in cells of the omentum with no sign of inflammatory cells about them. Six days after inoculation the bacilli which were seen in cells of the omentum have undergone multiplication, as indicated by great masses of bacilli arranged in parallel chains. There is still no sign of inflammatory reaction about them. After 10 days the large masses of bacilli are no longer found in the omental cells, having been included, possibly, in the newly formed tubercles. At 14 days the acid-fast bacilli, which are now found only in or about tubercles, stain faintly. Also at this period there appear about the larger tubercles minute granular structures which are sometimes acid-fast and sometimes non-acid-fast. After 17 days the tubercles and the tissues surrounding them contain many long, beaded bacilli which stain a bright red.

FURTHER STUDIES ON THE SURVIVAL TIME OF TUBERCULOUS RABBITS INJECTED WITH FERRIC CHLORIDE. Vally Menkin, Boston, Mass.

Abstract. Previous experiments have shown that repeated intravenous injections of ferric chloride (0.25 per cent) are followed by an accumulation of iron in the caseous areas of tuberculous animals. Concomitantly with this accumulation it was found in a series of 16 tuberculous rabbits that there was a marked increase in the survival time as compared with that in the controls. These studies have been repeated and extended with some modification of technique in the present series, consisting of 36 rabbits, with the following results:

1. The survival time of 10 control brindle rabbits ranged from 71 to 124 days with an average of 94 days. The survival time of 10 experimental brindle rabbits ranged from 89 to 190 days with an average of 135 days. The average increase in the experimental group was therefore 41 days, as compared with 48 days in the previous series.

2. The average weight plotted against time showed a slight increase in weight in the experimental animals during part of the period of ferric chloride injections. This was followed by a gradual fall in weight, terminating in the death of the last experimental rabbit the 28th week after inoculation with tubercle bacilli. The control group showed no average increase in weight during the period when the experimental animals were receiving ferric chloride injections. At the 10th week a sharp fall in weight began to take place, terminating in the death of the last of the control rabbits by the 17th week.

3. A comparison of the pathological appearance of the lungs revealed fewer tuberculous foci in the 4 shortest experimental survivors (89 to 112 days) than in the 4 shortest survivors of the control group (71 to 79 days).

4. In a parallel series of 24 tuberculous rabbits the latter were either killed at various intervals, or, in a few cases, allowed to die of their disease. This was done in order to compare the progress of the disease, as indicated by the extent of the pulmonary lesions, in the control and in the experimental rabbits injected repeatedly with ferric chloride, when both groups of animals had had the disease for about the same length of time. The interval between the injection of tubercle bacilli and the death of the animals ranged from 45 to 97 days. In 83 per cent of the animals, the tuberculous lesions in the lungs were distinctly less developed in the experimental group than in the control group. These results indicate that repeated ferric chloride injections retard the progress of tuberculous lesions in the lungs of rabbits infected with a bovine strain of tubercle bacilli.

5. In a short preliminary series of 15 tuberculous rabbits, 8 of which had received a total of 45 cc. of 0.25 per cent ferric chloride followed by a total of

It is evident also that abscesses and caseation, which is essentially a dead abscess, represent the more serious and dangerous lesions in normal animals and in human beings, and animals with increased resistance infected with bacilli of high virulence. It is the formation of these abscesses with their subsequent rupture that leads to cavity formation, hemorrhage and serious "spreading" of the disease. The participation of the neutrophil in the tuberculous process as the predominant cell type indicates the most serious type of infection. Bacilli are always abundant in such lesions except in the old caseous foci.

Discussion

(Dr. Max B. Lurie, Philadelphia.) In regard to the correlation which Dr. Medlar has brought out between the polynuclear leucocyte and the tubercle bacilli, I wish to say we have had the opportunity of following a strain of tubercle bacilli which is essentially the same type as Dr. Medlar has been using, and we have followed the number of bacilli present in the lesions, not by the number of tubercle bacilli which can be stained, but by the actual number of living bacilli present, as determined by culture, and it was found that the numbers of tubercle bacilli were greater before there was any infiltration of polynuclear leucocytes, and that with this particular strain of tubercle bacilli, a strain which grows with greatest rapidity in the rabbit body, the tubercle bacilli were greatly reduced in number when the first stages of caseation and infiltration of polynuclears set in. One reason for the appearance of the polynuclears in the lesion is the death of the cells and the release of the remaining contained bacilli.

(Dr. N. W. Popoff, Rochester, N. Y.) Have you noticed any definite relation between the type of cytological, experimentally produced change, and the picture of the blood?

(Dr. Medlar, closing.) The question of trying to determine from sections whether tubercle bacilli are living or not is of course impossible. All I can say is that during the first process where the monocyte is predominant, there is an increase of tubercle bacilli which causes disintegration of the monocytes. Subsequent to the degeneration of the monocytes there occurs an influx of the polynuclear leucocyte. Whether tubercle bacilli are killed before the polymorphonuclear leucocytes get in, I do not know, but certainly we get a tremendous increase of stainable bacilli when the sections are full of polymorphonuclears.

In regard to the question about the blood, I may say that for the last five years I have been doing a large number of leucocyte counts; we have had over 30,000 of them, and we have followed a great many tuberculous cases by weekly counts for a year or more. We have done studies on rabbits also. I have not seen any case which had progressive tuberculosis in which the neutrophil was not increased in the circulation. The percentage of neutrophils may be normal, but in such instances one may find a larger number of immature neutrophils in the circulation than of the mature.

CHANGES WHICH OCCUR IN THE TUBERCLE BACILLUS IN RELATION TO THE DEVELOPING TUBERCLE. C. Eugene Woodruff, Nashville, Tenn.

Abstract. Following intraperitoneal inoculation of tubercle bacilli in the guinea pig the bacilli collect in large part in the omentum. By studying "spread" inoculations of the omentum, stained by Ziehl-Neelsen's method, morphological changes in the bacilli have been identified. After 48 hours the bacilli are found bunched in certain cells — usually in polymorphonuclears or monocytes,

READ BY TITLE

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- SIMPLE GOITER PRODUCED IN RABBITS BY CABBAGE IN THE ABSENCE OF LIGHT.
Leon K. Baldauf and (by invitation) Anna Cipra, New York City.
- ATROPHY OF THE LIVER ASSOCIATED WITH HYPERTHYROIDISM. D. C. Beaver
(by invitation), Rochester, Minn.
- THE RELATION OF HEMOPOIETIC TUMORS TO MULTIPLE MYELOMAS AND TO
EWING'S SARCOMA. C. L. Connor, San Francisco, Calif.
- THE MECHANISM OF THE PRESSOR ACTION OF DIMETHYLGUANIDINE SULPHATE.
Harry Goldblatt and Howard T. Karsner, Cleveland, O.
- UREA CLEARANCE IN NEPHROPATHIC DOGS. Ramon F. Hanzal (by invitation),
Harry Goldblatt and (by invitation) Ward W. Summerville, Cleveland, O.
- SYPHILIC PULMONARY MESAORTITIS. Howard T. Karsner, Cleveland, O.
- AN ANALYSIS OF 104 CASES OF CANCER OF THE LARGE INTESTINE. Howard T.
Karsner and (by invitation) Burton Clark, Jr., Cleveland, O.
- MICROINCINERATION STUDIES OF HUMAN CORONARIES. D. Y. Ku (by invita-
tion), Woosung, China.
- THE ETIOLOGY OF BRAIN ABSCESS ACCOMPANYING CHRONIC PULMONARY SUP-
PURATION. Howard A. McCordock, St. Louis, Mo.
- MESENTERIUM COMMUNE WITH INTESTINAL OBSTRUCTION. Alan R. Moritz,
Cleveland, O.
- UREA CLEARANCE IN NORMAL DOGS. Ward W. Summerville, Ramon F.
Hanzal (by invitation) and Harry Goldblatt, Cleveland, O.

22 cc. of 5 per cent CaCl_2 , only a relatively slight increase in the survival time was found in the experimental group of tuberculous rabbits amounting to about 27 per cent over that of the controls. Further studies are now in progress on this phase of the problem.

6. *In vitro* studies show that when to a saline suspension of Ravenal bovine tubercle bacilli 0.25 per cent of ferric chloride is added, a flocculent yellowish precipitate (probably a ferric proteinate) sinks to the bottom of the test tube, leaving a clear supernatant fluid.

The data in the published and in the present studies of tuberculous rabbits repeatedly injected intravenously for 10 and 20 weeks respectively with 0.25 per cent ferric chloride show that concomitantly with an accumulation of iron in caseous areas there is a retardation in the development of tuberculous lesions in the lungs, as well as a significant increase in the survival time of experimental animals.

Discussion

(Dr. E. M. Medlar, Mt. McGregor.) I should like to ask whether careful histological studies were made of any of the lesions, because if this treatment has a beneficial effect on the tuberculous lesions, certainly the lesions from animals so treated should be very different histologically from those in the untreated animals.

(Dr. C. A. Doan, Columbus.) Were any cytological studies of the blood, particularly of the red cells, made? There is a definite decrease in the color index in chronic tuberculosis, and I wonder if these injections of iron had any influence upon this phenomenon in the peripheral circulation itself.

(Dr. Menkin, closing.) In answer to Dr. Medlar's question, we have not yet studied these lesions histologically in a very extensive manner, but in some cases that we have studied, however, the type of tubercle is very much like those shown here to-day, with the usual caseation in the center, and mononuclear phagocytic response at the periphery. We were interested to know whether there would be a greater fibroblastic proliferation about the lesions, but I was unable to find anything very consistent; some lesions showed at their periphery a great deal of vascular engorgement, but we also found that in some of the controls.

In reference of Dr. Doan's question, I think that the results of our experiments indicate that concomitantly with the accumulation of iron in tuberculous areas there is a prolongation in the survival time of experimental animals, but it is conceivable at the same time that there may be other factors concerned in explaining the effects on the course of the disease besides the accumulation of iron in tuberculous foci. I have done hemoglobin determinations and total leucocyte counts for several weeks on two rabbits, and I have found no appreciable change, but I have as yet made no studies of the color index.

THE PERSISTENCE OF TUBERCULOUS INFECTIONS. H. E. Robertson, Rochester, Minn.

Abstract. Routine studies of tuberculous foci examined by stained sections show a surprising number in which signs of latent or persistent activity of the infection are indubitably present. In many such cases there has never been even a suspicion of the disease during life. The study strengthens the assumption "once infected (with tuberculosis) always infected," which is a safe but probably not a wholly true generalization.

For purposes of this study endocarditis has been classified as follows:

- I. Rheumatic endocarditis
- II. Bacterial endocarditis
 - A. Primary bacterial endocarditis
 - (a) Acute
 - (b) Subacute
 - B. Secondary bacterial endocarditis

Rheumatic endocarditis is defined anatomically as the type in which the vegetations are all small and firm and incapable of forming emboli. The diagnosis is based entirely on the structure of the vegetations. No case was excluded because of failure to demonstrate Aschoff bodies in the myocardium. Bacterial endocarditis includes all instances in which the vegetations are soft and friable. Cases in which lesions of both types are present have been classified as bacterial endocarditis.

Only active rheumatic endocarditis has been studied. This is regarded as acute when there is no scarring of the leaflets, and recurrent when the leaflets show fibrous thickening from a former infection. Old healed rheumatic valves, that is, those without fresh vegetations, have been excluded from this study.

Bacterial endocarditis is divided into a "primary" type which seems to begin as a bacteremia, and a "secondary" type in which the invasion of the blood stream is obviously secondary to a previously established infection such as acute endometritis.

The distinction between acute and subacute endocarditis is arbitrary. Thayer² grouped all cases with a duration of less than one month as acute, but we have used a duration of six weeks as an arbitrary line of separation. The duration indicated in the tables is the estimated period in which the infective process was present, as indicated by chills, fever, positive blood cultures, enlargement of the spleen, embolic processes, and so on. But when a patient with cardiac decompensation from an old valvular defect develops acute endocarditis it is frequently impossible to determine the time of onset of the infectious process unless a thorough clinical study has been made. For this reason there is, no doubt, some error in the separation of the acute and subacute groups.

It would have been better to subdivide bacterial endocarditis still farther on the basis of etiology, as streptococcic, pneumococcic,

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GLOMERULAR LESIONS ASSOCIATED WITH ENDOCARDITIS *

E. T. BELL, M.D.

(From the Department of Pathology, University of Minnesota, Minneapolis, Minn.)

Since Löhlein's publication in 1910,¹ it has been known that focal glomerular lesions may occur in the course of subacute bacterial endocarditis. The purpose of this investigation is to study in detail the pathogenesis of the focal lesions and also to describe other glomerular lesions associated with endocarditis that have heretofore received little or no attention.

The material available for study consisted chiefly of small pieces of kidneys preserved in formalin or tissues embedded in paraffin. Paraffin sections were cut and stained with hematoxylin and eosin, and azocarmine. Tissues preserved for ten years or longer in formalin stain fairly well with azocarmine if they are given a preliminary treatment with ammonia water and are refixed in Zenker's or Helly's fluid. Material secured fresh during the course of this study was usually fixed in Helly's fluid. The best results are obtained when fixation is accomplished by injection of the fixing fluid into the renal artery. In calculating the percentage of the various types of glomerular lesions, 100 or more glomeruli were examined. In most instances, however, only one block of tissue was available and it is recognized that this is a possible source of error. When the percentage of involved glomeruli is high, embolic lesions are found in all sections examined, but when the percentage is low, some sections show no embolic lesions. It is therefore possible that the study of numerous sections from various parts of the kidneys would have revealed some embolic lesions in the group that are recorded as negative.

* This investigation was aided by a grant from the Ella Sachs Plotz Foundation.
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TABLE I

*Acute Bacterial Endocarditis **

Number	Age	Sex	Duration	Old valvular defect	Blood culture	Weight of spleen	Glomerular lesions		
							Infarcts	Embolic	Diffuse
	<i>years</i>					<i>gm.</i>			
14-209.....	42	M	1 wk.	+	o	325	+	-	+
15-391.....	43	M	3 wk.	-	o	153	-	-	-
16-54.....	35	M	2 wk.	-	o	762	+	-	-
16-128.....	19	F	5 days	+	o	350	-	-	-
17-31.....	19	M	3 wk.	+	s.h.	465	+	-	-
17-213.....	43	F	2 wk.	-	o	325	-	-	-
19-197.....	34	F	6 days	+	o	520	+	-	-
20-2.....	18	M	5 wk.	+	o	280	+	-	-
21-124.....	78	M	4 wk.	+	o	130	+	-	-
22-148.....	19	F	1 mo.	-	o	270	+	-	+
25-600.....	38	F	10 days	+	o	375	-	-	-
25-1022.....	41	F	1 mo.	+	o	?	+	-	-
26-600.....	2	F	1 mo.	-	s.v.	60	-	-	-
26-635.....	52	M	?	-	o	250	-	-	+
26-818.....	25	M	4 days	-	o	190	+	-	-
27-306.....	24	F	3 days	+	o	300	+	a	-
27-479.....	33	F	1 mo.	-	o	155	+	-	+
27-847.....	12	F	10 days	-	st.	55	-	-	-
27-1008.....	58	F	1 mo.	+	o	210	+	-	-
28-185.....	16	F	1 wk.	-	st.	400	+	a	-
28-186.....	39	M	2 days	+	s.	275	-	a	-
28-343.....	26	M	1 mo.	+	s.v.	420	+	-	+
28-481.....	18	M	5 wk.	-	o	550	-	-	-
28-520.....	57	M	5 days	+	o	310	+	+	-
28-1251.....	34	F	?	-	o	160	-	-	-
28-1276.....	55	M	?	-	o	215	-	-	+
28-1347.....	30	M	?	-	o	85	+	-	-
28-1348.....	64	M	3 wk.	-	s.v.	300	+	-	-
28-1628.....	21	M	5 wk.	-	s.v.	340	+	-	+
29-736.....	31	M	6 days	-	o	200	-	a	-
29-824.....	37	F	3 wk.	-	pn.	200	-	-	+
29-1229.....	24	F	1 mo.	-	-	340	+	-	+
29-1278.....	42	M	6 days	-	o	660	+	-	+
29-1307.....	26	M	10 days	-	s.h.	425	+	+	-
29-1368.....	55	F	1 mo.	+	o	140	+	-	-
29-1387.....	44	F	2 wk.	+	o	475	+	+	-
29-1406.....	29	F	3 wk.	-	o	100	+	-	-
29-1441.....	21	F	1 mo.	-	st.	350	+	-	-
29-1533.....	72	F	?	+	o	90	+	-	-
29-1574.....	46	M	1 mo.	-	o	94	+	-	+
29-1893.....	69	F	1 mo.	-	s.v.	290	-	-	-
30-85.....	52	F	?	+	o	225	-	-	-
30-226.....	27	M	5 wk.	+	o	325	-	-	-

* Under embolic lesions "a" indicates that glomerular abscesses were present. Under blood culture "o" indicates that no blood culture was made; s.h. *Streptococcus hemolyticus*; s.v. *Streptococcus viridans*; a. streptococcus of undetermined type; st. staphylococcus; pn. pneumococcus.

staphylococcic, gonococcic and influenzal, but the bacterial studies of our material are too meager to be of much value in this direction. Various investigations indicate that streptococci are responsible for nearly all cases of subacute endocarditis and a large proportion of the acute cases. There is no evidence that the streptococci found in the acute type are different from those in the subacute form. Endocarditis, caused by organisms other than streptococci, is nearly always of acute type, but there are occasional exceptions. Acute and subacute endocarditis cannot be separated satisfactorily on an etiological basis.

I. RHEUMATIC ENDOCARDITIS

The kidneys from 104 cases of active rheumatic endocarditis have been examined. In every instance fresh rheumatic vegetations were found on the valve leaflets. In 53 cases there was no evidence of old valvular disease, and the lesion was therefore regarded as acute. In 51 cases the lesion was regarded as a recurrent acute infection because of the presence of old scar tissue in the leaflets. There is no significant difference in the incidence of glomerular lesions in the acute and recurrent forms. In 81 of the 104 cases (77.8 per cent) the glomeruli are either entirely normal or they show only a slight alteration. In one instance a typical advanced chronic diffuse glomerulonephritis was present. In the remaining 22 cases (21 per cent) the glomeruli show a moderate but definite acute diffuse inflammatory reaction. Nearly all of the glomeruli are involved. There is a definite increase in the number of endothelial cells and their cytoplasm has become conspicuous. The capillary basement membrane is occasionally thickened. The appearance is such as is shown in Figure 1. Occasionally there is also a notable increase in the number of leukocytes in the glomerular capillaries.

The increase of endothelium is much less prominent and the capillary obstruction much less pronounced than in clinical acute glomerulonephritis. There are no intracapillary fibers but in other respects the lesion differs only in degree from the clinical case. It is probable, however, that lesions of this moderate degree readily return to normal and do not often progress to the extent of complete capillary obstruction.

In 2 of the 22 cases the endothelial proliferation is very prominent

valvular defects with cardiac decompensation, and the presence of active endocarditis was not recognized clinically. In the other 6 cases the records are incomplete, but the duration was probably less than six weeks. Old valvular defects were present in 15 of the 56 cases (27 per cent). Blood cultures are recorded in only 16 cases. Of these, 6 showed *Streptococcus viridans*, 2 *Streptococcus hemolyticus*, 1 streptococcus of undetermined type, 3 staphylococcus, 1 pneumococcus, and 3 were negative.

The average weight of the spleen in 52 adults was 316 gm. The weight was 400 gm. or more in 13 instances. Infarction of the spleen or kidneys was present in 34 instances (61.7 per cent).

Glomerular lesions of embolic type were found in only 4 cases. In 3 of these, only small fresh intracapillary thromboses were found, 2 to 6 per cent of the glomeruli being involved. In the fourth case (29-1387), 3 per cent of the glomeruli showed small focal fibrous lesions, but no fresh lesions were found. The fibrous lesions may have resulted from an active endocarditis some time previous to the terminal attack, since an old valve defect was present.

There were 16 instances of acute diffuse glomerulitis, 2 of which were of exudative type and the others proliferative. Glomerular abscesses were found in 4 instances.

(b) *Subacute Cases*: (Table II.) Sixty-seven of the 108 cases (62 per cent) of this group were examples of active endocarditis on leaflets thickened by a previous rheumatic infection. In 46 cases no blood culture was made. In the remaining 62, 11 were negative, 28 showed *Streptococcus viridans*, 7 *Streptococcus hemolyticus*, 14 streptococci of undetermined type, and 2 cocci of undetermined type. The negative cultures were chiefly from cases in which only one blood culture was made.

Secondary anemia of some degree was found in almost every instance in which the blood was examined. The average weight of 101 spleens was 419 gm. The weight was 400 gm. or more in 43.5 per cent, and 600 gm. or more in 19.4 per cent of the cases. Infarction of the spleen or kidneys occurred in 69.4 per cent, only a little above the incidence of infarction in the acute group (Table IV).

In 19 of the 108 cases studied (17.6 per cent) all the glomeruli were either entirely normal, or they showed only minor alterations, such as a slight increase in the prominence of the endothelium or a little uneven thickening of the capillary basement membrane. They were

TABLE I (continued)

Number	Age	Sex	Duration	Old valvular defect	Blood culture	Weight of spleen	Glomerular lesions		
							Infarcts	Embolic	Diffuse
	<i>years</i>					<i>gm.</i>			
30-589.....	53	F	?	+	o	300	-	-	-
30-1465.....	12	F	1 wk.	+	o	450	-	-	+
30-1655.....	61	M	2 wk.	-	o	314	-	-	-
30-1776.....	17	F	?	-	o	275	+	-	-
31-99.....	3½	M	8 days	+	o	100	-	-	-
31-554.....	39	M	1 wk.	+	o	175	+	-	+
31-579.....	50	F	1 mo.	-	-	710	+	-	+
31-708.....	15	F	3 wk.	+	o	344	+	-	-
31-939.....	42	F	5 wk.	-	o	133	+	-	-
31-1032.....	45	M	?	+	o	400	+	-	+
31-1193.....	26	F	?	-	o	375	-	-	-
31-1369.....	30	M	?	+	s.v.	250	-	-	-
31-1762.....	51	M	3 wk.	-	-	685	+	+	+

but still less pronounced than in a clinical case of acute glomerulonephritis.

In 3 of the 22 cases with acute diffuse glomerulitis, lesions of embolic type were also found in the glomeruli. The number of glomeruli involved was 1, 10 and 25 per cent respectively in the 3 cases. The lesions were all intracapillary thromboses. These will be described later, since they are similar to the small fresh lesions of subacute bacterial endocarditis.

There is no evident relation between acute glomerulitis and associated terminal infections. It is just as frequent in those dead of cardiac decompensation as in those with terminal pneumonia, peritonitis, and so on.

II. BACTERIAL ENDOCARDITIS

A total of 233 cases has been studied, of which 164 were primary and 69 secondary. Of the 164 primary infections, 56 were classed as acute and 108 as subacute.

A. Primary Bacterial Endocarditis

(a) *Acute Cases:* The 56 cases of this group are listed in Table I. It will be noted that the duration of the infective process was not known accurately in 11 instances. Five of these patients had old

TABLE II

Subacute Bacterial Endocarditis *

Number	Age	Sex	Duration	Old valvular defect	Blood culture	Weight of spleen	Glomerular lesions						Comment
							Infarcts	Embolus		Diffuse	Crescents	Normal	
								Hyaline	Fibrous				
11-76.....	years 25	M	3 mo.	-	o	?	-	o	o	100	25	o	Chronic glomerulonephritis
12-131.....	33	M	3 mo.	+	-	420	-	o	o	100	10	o	Acute glomerulonephritis
13-165.....	33	M	3 wk. +	+	-	595	-	o	o	100	o	o	
13-190.....	35	F	?	+	s.	296	-	o	o	100	o	o	
14-49.....	25	M	3 wk. +	+	o	very large	+	o	o	100	o	o	Acute glomerulonephritis
15-97.....	29	F	9 wk.	-	o	large	+	o	o	100	o	o	Acute glomerulonephritis
16-89.....	30	M	?	+	s.	594	-	4	7	4	85	o	Acute extracapillary glomerulonephritis
16-124.....	45	M	5 mo.	-	s.v.	325	+	5	5	100	o	o	
16-203.....	61	M	?	+	o	220	-	2	8	o	o	90	
16-413.....	38	M	3 mo.	+	o	600	+	2	10	o	3	85	
17-36.....	29	F	6 mo.	+	s.v.	260	+	4	20	o	o	76	
17-174.....	28	M	?	+	-	340	-	o	o	o	o	100	
17-202.....	25	M	1½ yr.	+	o	1400	+	o	o	100	o	o	Chronic glomerulonephritis
17-260.....	25	M	7 mo.	+	s.h.	650	+	25	50	25	6	o	
18-102.....	23	M	3 mo.	-	o	440	+	o	o	100	o	o	
18-122.....	30	M	3½ mo.	-	o	680	+	o	o	100	o	o	Acute glomerulonephritis
18-175.....	34	M	8 mo.	-	o	500	-	9	52	84	9	o	
17-28.....	29	M	1 yr.	+	s.h.	360	+	o	o	100	o	o	
19-141.....	55	M	6 wk.	-	-	325	+	5	o	100	o	o	
19-161.....	23	F	8 mo.	-	s.v.	285	+	10	86	4	o	o	Uremia
19-264.....	29	M	?	-	o	365	-	o	o	100	10	o	Chronic glomerulonephritis
19-276.....	16	M	?	+	s.h.	200	+	o	o	100	o	o	

classified as normal unless the alterations were definite, as shown in Figure 1. The group with normal glomeruli show no distinctive difference in the duration of the disease, but there is evidence that cardiac decompensation was a more important factor than septicemia in causing death. The average weight of the spleen in these 19 cases was 291 gm., as compared with 419 gm. for the entire group, and 449 gm. for the group in which glomerular lesions were present. In a general way, the size of the spleen depends upon the duration and the intensity of the infective process, and it may be inferred that glomerular lesions depend upon these same factors in the disease.

Diffuse glomerulitis was found in 64.8 per cent, with embolic lesions in 35.2 per cent, and alone in 29.6 per cent. Embolic lesions were found in 52.8 per cent, with diffuse glomerulitis in 35.2 per cent, and alone in 17.6 per cent. In the group with embolic lesions only, the average weight of 17 spleens was 331 gm., as compared with an average of 480 gm. in those with diffuse glomerulitis. There were 3 very large spleens in the purely embolic group, but there is a suggestion that the septicemia was less intense than in those with diffuse glomerulitis.

It is to be noted that both embolic and diffuse glomerular lesions were present in 35.2 per cent. Very frequently a glomerulus showed an embolic lesion in one lobule and diffuse glomerulitis in the others. The lesions did not exclude one another except when the embolic lesion was very large, and when the glomerulitis was as pronounced as in clinical glomerulonephritis.

B. Secondary Bacterial Endocarditis

Sixty-nine examples of this group were studied (Table III). Endocarditis was always a distinctly secondary process and was never recognized clinically. It was not considered the cause of death in any instance, and was presumably a terminal infection. It was associated with definite infectious processes in 42 instances, of which the most frequent were acute endometritis (14), and lobar pneumonia (9). In these the duration given in the table is that of the major illness and therefore it represents the maximum possible duration of the endocardial disease. In 27 instances endocarditis appeared as a terminal complication of some non-infective process,

TABLE II (continued)

Number	Age	Sex	Duration	Old valvular defect	Blood culture	Weight of spleen gm.	Glomerular lesions						Comment
							Embolie			Diffuse	Cres- cents	Normal	
							Infarcts	Hyaline	Fibrous				
26-639....	17	M	3 mo. +	+	COC.	400	+	0	100	0	0	Acute glomerulonephritis	
26-654....	34	M	6 mo. +	+	-	1040	+	0	100	0	0		
26-760....	55	M	?	+	0	650	-	5	61	0	34		
26-859....	38	F	4 mo. ?	+	0	150	-	0	0	0	100		
26-1082....	43	M	6 mo.	+	0	875	+	0	100	0	0		
26-1136....	46	M	3 mo.	+	s.v.	500	-	0	100	0	0		
27-59.....	12	F	2 mo.	-	0	175	-	0	0	0	100		
27-535....	26	M	9 mo.	+	s.	180	+	12	77	0	4	Uremia	
27-749....	34	M	1 yr.	+	0	540	+	0	0	0	100		
27-768....	44	M	8½ mo.	+	0	820	+	0	100	0	100		
27-795....	25	F	9 mo.	+	0	400	+	0	0	0	0		
27-861....	29	M	10 wk.	-	s.v.	840	+	0	100	0	0		
27-1280....	34	M	7 wk.	+	s.	375	+	0	100	0	0		
28-1.....	48	F	6 wk.	+	s.h.	200	+	40	99	3	1		
28-74.....	31	F	2 mo.	+	s.	620	+	0	100	0	0		
28-91.....	35	M	?	+	0	600	+	1	100	0	0		
28-315....	39	M	7 mo.	+	0	400	+	0	0	0	100		
28-320....	29	M	3 mo.	+	0	100	+	0	0	0	100		
28-1098....	30	F	5 mo.	-	s.v.	450	-	5	100	0	0		
28-1234....	42	M	2 mo.	+	s.h.	320	-	0	100	0	0		
28-1243....	46	M	7 mo.	-	-	400	+	3	0	0	94		
29-137....	60	M	6 mo.	-	s.	450	+	0	100	0	0		
29-449....	35	F	1 yr.	-	0	350	+	18	65	5	0		
29-746....	63	M	5 mo.	-	-	425	+	0	100	0	0		
29-1085....	26	F	3 mo.	-	s.v.	250	+	26	0	5	61		

Acute glomerulonephritis
Uremia

20-88....	51	M	6 wk.	-	0	314	+	0	0	100	0	0
20-165....	43	M	6 mo.	+	0	325	+	0	0	100	0	0
20-296....	16	M	7 wk.	-	-	700	+	0	0	100	0	0
20-344....	12	F	5½ mo.	-	0	190	+	10	66	0	24	0
20-368....	18	M	6½ mo.	-	s.v.	310	+	34	17	35	24	0
21-45....	55	M	4 mo.	-	s.v.	310	+	27	8	8	10	48
21-283....	24	M	7 mo.	-	s.v.	400	+	2	35	63	5	0
21-414....	50	M	2 mo.	+	0	large	+	0	2	0	0	98
21-468....	36	M	6 mo.	-	-	800	+	2	51	0	20	26
21-513....	42	M	10 wk.	+	s.v.	170	-	2	0	0	0	100
22-554....	43	M	5 mo.	-	0	310	-	2	67	0	12	19
23-390....	45	M	?	+	0	480	-	90	0	100	10	0
23-508....	76	M	7 wk.	+	0	360	-	2	22	0	0	76
24-4....	30	M	5 mo.	-	s.v.	?	+	37	0	95	3	0
24-91....	39	M	3 mo. ?	+	s.	670	+	6	7	90	0	10
24-121....	59	M	8 mo. ?	+	coc.	400	-	0	0	0	0	100
24-241....	23	F	3 mo.	+	s.v.	240	+	5	28	0	0	67
24-605....	35	M	3 mo. +	+	-	730	+	6	34	50	20	20
24-769....	14	F	7 mo.	+	s.v.	135	+	8	44	0	30	20
24-770....	10	F	5 mo. +	+	s.v.	325	+	11	21	70	0	0
25-142....	4	F	6 wk.	-	s.	80	+	6	0	0	0	94
25-362....	42	M	1 mo. +	-	s.v.	280	-	0	0	10	0	90
25-589....	30	F	2 mo. +	-	0	700	+	5	0	87	7	0
25-789....	45	M	6 wk. +	+	-	135	+	5	0	0	0	95
25-565....	56	M	11 mo.	-	0	170	-	0	0	0	0	100
25-630....	30	M	3 mo.	+	s.v.	700	-	0	0	100	0	0
25-888....	35	F	6 wk.	-	s.h.	445	+	2	0	100	0	0
25-1031....	52	M	2 mo.	+	s.	160	+	0	0	0	0	100
26-191....	17	F	8 mo.	+	0	300	+	6	17	97	1	0
26-201....	46	M	3 mo.	+	s.v.	625	+	13	14	73	1	0

Uremia
Uremia

* Under blood culture "0" indicates that no culture was made; s.v. *Streptococcus viridans*; s.h. *Streptococcus hemolyticus*; s. *Streptococcus* of undetermined type; coc. coccus of undetermined type.

TABLE III

Secondary Acute Bacterial Endocarditis *

Number	Age	Sex	Duration	Old valvular defect	Blood culture	Weight of spleen gm.	Glomerular lesions			Cause of death
							Infarcts	Embolie	Diffuse	
13-180.....	63	M	2 mo.	+	o	small	-	-	-	Carcinoma of stomach
14-255.....	39	M	4 wk.	+	o	285	-	-	+	Lobar pneumonia
16-89.....	30	M	2 wk.	+	s.	594	-	+	-	Erysipelas
16-358.....	62	M	?	-	o	75	-	-	-	Peritonitis, postoperative
17-233.....	38	F	?	-	o	200	-	-	-	Abdominal tumor
18-39.....	39	M	?	-	-	160	+	-	-	Pernicious anemia
18-53.....	47	M	?	+	o	130	-	-	-	Carcinoma of stomach
19-94.....	46	F	9 days	-	s.h.	125	+	-	-	Peritonitis, postoperative
20-122.....	63	M	?	-	-	235	+	-	-	Postoperative shock
20-209.....	56	M	?	-	s.h.	300	+	-	+	Carcinoma of stomach, peritonitis
20-267.....	25	F	?	-	o	?	+	-	+	Acute endometritis
20-388.....	38	F	?	-	o	810	+	-	-	Leukemia
20-428.....	65	F	1 wk.	-	o	230	+	-	-	Pneumonia
21-150.....	21	F	4 wk.	-	-	120	-	-	-	Acute endometritis
21-165.....	65	M	1 mo.	-	o	65	-	-	-	Carcinoma of stomach
21-237.....	36	F	3 wk.	-	s.h.	310	+	-	+	Acute endometritis
21-307.....	54	M	10 days	-	-	220	-	-	-	Primary hypertension
21-559.....	28	M	14 wk.	+	s.v.	140	-	-	+	Ulcerative colitis
22-134.....	46	F	9 days	-	s.h.	500	-	-	-	Influenzal pneumonia
22-149.....	68	M	?	-	o	80	-	-	-	Carcinoma of prostate
22-229.....	23	F	?	-	o	140	-	-	-	Peritonitis
24-566.....	26	M	?	-	pn.	425	+	-	-	Thrombosis of cavernous sinus
24-628.....	51	M	?	-	o	?	-	-	-	Appendicitis
24-807.....	20	F	7 mo. ?	+	s.h.	225	+	+	+	Lupus erythematosus, acute disseminated
25-103.....	68	M	3 wk.	-	o	205	+	-	-	Multiple fractures
25-1019.....	28	M	2 wk.	-	o	225	-	-	-	Lobar pneumonia
26-565.....	20	F	6 wk.	-	s.h.	170	-	-	-	Acute endometritis
26-1134.....	43	M	?	-	o	1180	-	-	+	Leukemia, tricuspid valve
26-1154.....	38	M	?	-	o	140	-	-	+	Inguinal granuloma
27-149.....	21	F	8 days	-	o	160	-	-	-	Peritonitis
27-177.....	57	M	4 days	-	o	250	+	-	-	Hypertension
27-392.....	27	F	?	-	s.v.	250	-	-	-	Sinus thrombosis

usually of chronic nature. The duration of the endocarditis in this group could seldom be determined. There were 8 cases of carcinoma of the stomach.

In 59 instances there had been no previous valvular disease, but in 10 the vegetations formed on old healed rheumatic valves. Blood cultures, which were made in only 20 cases, showed *Streptococcus hemolyticus* 10, *Streptococcus viridans* 2, streptococcus 1, pneumococcus 2, staphylococcus 2, negative 3.

The average weight of 59 adult spleens, not including leukemia, was 307 gm. Seventeen of these weighed over 400 gm. The largest spleens were found in association with acute endometritis, the average weight being 448 gm. The rôle of infection in causing enlargement of the spleen is obvious. The average weight of the adult spleen in 21 instances where endocarditis was a terminal complication of a chronic disease was 207 gm., exclusive of leukemia. In 38 cases where death was due entirely to an acute infectious process, the average weight of the spleen was 363 gm.

Acute diffuse glomerulitis was found in 23 instances (33.3 per cent), 17 times in association with definite infectious processes and 6 times with chronic diseases. The average weight of the spleen in the cases with glomerular lesions was 352 gm., and in those without glomerular lesions 267 gm., but there were several very large spleens in cases without glomerulitis.

Embolic glomerular lesions were found in 4 cases. Infarction of the spleen or kidneys was present in 30 cases (43.4 per cent) and absent in 39.

A summary of some of the data reviewed in the preceding paragraphs is given in Table IV.

DIFFUSE GLOMERULITIS

This lesion was evidently recognized by Löhlein in 1910, since he noted that the capillary loops of many glomeruli were thicker and broader, the nuclei increased above the normal, and the lumens of the capillaries apparently filled with protoplasm. The lesion consists chiefly in an increase in the size and number of the endothelial cells, but there is also a thickening of the capillary basement membrane in many instances. The glomeruli are not enlarged, but they have an avascular solid appearance. The lumens of the

acute endocarditis the average weight of the spleen is much greater (449 gm.) in cases where glomerular lesions are present than in those with normal glomeruli (291 gm.). In both forms of acute bacterial endocarditis the spleens are smaller and the incidence of glomerulitis is less than in the subacute type. Both the duration and the intensity of the infection are factors in the development of glomerulitis. Glomerulitis is more frequent than embolic lesions in subacute endocarditis.

Clinical acute glomerulonephritis in association with endocarditis is interpreted as the result of the infection responsible for the endocarditis, but chronic glomerulonephritis is evidently due to an antecedent infection. A contracted kidney cannot develop in the comparatively short course of endocarditis.

THE EMBOLIC LESIONS

The embolic lesions were described by Löhlein in 1906³ and 1910.¹ Baehr⁴⁻⁸ has made important contributions to this subject in a series of papers published from 1912 to 1923. In his first communication, Löhlein interpreted the lesions as capillary thromboses, but in his second paper he explained them as small infarcts resulting from the lodgement of minute infected emboli detached from the valves of the heart. Both Baehr, and Fahr,⁹ agree with this latter interpretation.

Löhlein found typical embolic lesions in each of 8 cases of "chronic ulcerative endocarditis," but noted great variations in the number of glomeruli involved. In 3 of his cases *Streptococcus viridans* was obtained in blood culture.

Baehr^{4, 5} believed that only subacute streptococcus endocarditis is associated with embolic lesions. He found typical lesions in 21 of 25 such cases in which the blood culture was positive for *Streptococcus viridans*, and in 6 of 7 cases with negative blood cultures. The percentage of involved glomeruli varied from 2 to 75 in the group. No lesions were found in 1 instance of influenzal and another of gonorrheal endocarditis. In 54 instances of acute endocarditis "due to ordinary pathogenic bacteria" no embolic lesions were found. Baehr seems to distinguish "acute" from "subacute" endocarditis by the kind of organism obtained in blood culture rather than by the duration of the disease. In 75 cases of verrucous

capillaries are decreased in size by encroachment of the endothelial cells and the basement membrane (Fig. 1). Sometimes the thickened basement membrane is more responsible than the endothelium for this appearance. Occasionally a few polymorphonuclear leukocytes are found in the capillaries. The lesion is not identical with clinical acute glomerulonephritis, since the capillary obstruction is much less pronounced and no intracapillary fibers are found, but the difference is chiefly in the intensity of the reaction. In association with subacute endocarditis (Table II) there were 7 examples of typical acute glomerulonephritis, and there were 2 other cases in which the lesion was nearly as prominent as in clinical acute glomerulonephritis. It may be safely inferred that the moderate

TABLE IV

A Comparison of the Four Forms of Acute Endocarditis with Respect to Glomerular Lesions, Size of Spleen, and Frequency of Infarction of the Spleen and Kidneys

Endocarditis	Number examined	Embolic lesions	Diffuse glomerulitis	Normal	Weight of spleen	Infarcts
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>gm.</i>	<i>per cent</i>
Rheumatic	104	2.9	22.2	77.8	0	0
Acute primary bacterial...	56	7.1	28.6	66	316	61.7
Subacute bacterial	108	52.8	64.8	17.6	419	69.4
Secondary acute	69	5.8	33.3	63.7	307	43.4

diffuse glomerulitis, found so frequently with endocarditis, is a true glomerulonephritis differing only in intensity from the clinical form. Symptoms do not develop unless capillary obstruction is fairly pronounced.

Usually diffuse glomerulitis involves nearly all of the glomeruli, but in a few instances only a small proportion of them are affected. When associated with embolic lesions the lobules not involved in the embolism commonly show the characteristic diffuse reaction.

The causative factor in diffuse glomerulitis is evidently infection. Glomerulitis occurs in all forms of endocarditis but is much more frequent in the subacute form. Presumably its greater frequency in this group is due to the more prolonged and more severe septicemia. It was pointed out in a preceding paragraph that in sub-

forms of endocarditis, in endocarditis due to organisms other than streptococci, and even in the absence of endocarditis of any kind.

What are the factors which influence the formation of embolic lesions in bacterial endocarditis? It is obvious that the duration of the infection is of great importance, since the lesions are rare in the acute cases of less than six weeks duration and frequent in those of longer standing (see Table IV); but beyond the six weeks period no definite influence of the time element can be made out. The lesions are just as frequent at two or three months as they are after six months. They are usually caused by *Streptococcus viridans*, but may be produced by *Streptococcus hemolyticus* and other organisms. Staphylococcic infections, however, cause glomerular abscesses and not embolic lesions of the type under discussion. There is no evident relation to gross infarction in the spleen and kidneys, since infarction is nearly as frequent in the acute as in the subacute group (Table IV). However, in the subacute group embolic lesions were found in 57 per cent of those with infarcts and in 35 per cent of those without infarcts. The vegetations on the valves are commonly larger and more numerous in subacute than in acute endocarditis, and we would therefore expect a smaller number of involved glomeruli in the latter, but not a complete absence of the lesions, since gross infarction occurs readily. The fact that a certain duration of the infection is necessary suggests that immune bodies may play a rôle in the formation of the glomerular lesions. We do not understand why cases of subacute bacterial endocarditis that are similar in clinical and anatomical features show variations from 0 to 99 per cent in the number of glomeruli with embolic lesions.

THE STRUCTURE OF THE GLOMERULAR LESIONS

Two distinct types of lesions are found in embolic glomerulonephritis, the fresh or hyaline, and the fibrous. In sections stained with hematoxylin-eosin the hyaline lesions take a deep eosin stain and appear homogeneous, while the fibrous lesions stain less intensely with eosin and have a fibrous structure. In such preparations one gets the impression that the hyaline lesions are gradually transformed into the fibrous. Bachr came to this conclusion and interpreted the hyaline as recent and the fibrous as old lesions. The azocarmine stain, however, shows a sharp contrast between

(rheumatic) endocarditis Baehr and Sacks⁸ found no embolic lesions, but they mentioned 2 cases with thromboses of capillary loops.

Miller and Branch¹⁰ described typical embolic lesions in a case of endocarditis of fifty days duration caused by a hemophilic bacillus resembling *B. influenzae*.

In our experience, embolic lesions are not limited to subacute *Streptococcus viridans* endocarditis. They may be caused by *Streptococcus hemolyticus*, and they are also found in acute endocarditis. In 3 of 104 cases of rheumatic endocarditis capillary thromboses were found which correspond to the early stages of the embolic lesions. In 4 of 56 cases of acute endocarditis (duration of less than six weeks), and in 4 of 69 cases of secondary endocarditis embolic lesions were found. Most of these were small capillary thromboses, but some were typical large embolic lesions. In our series of 108 cases of subacute bacterial endocarditis embolic lesions were found in only 52.8 per cent. This is a surprisingly low figure in comparison with Baehr's observations, but it is a much larger series and includes all cases which came to autopsy from 1911 to 1932. The percentage of involved glomeruli varied from 2 to 99 per cent. It was over 50 per cent in 18, and 5 per cent or less in 8 cases.

In 1923 Baehr mentioned 2 instances in which embolic glomerulonephritis occurred in the absence of endocarditis. Fahr⁹ also recorded a similar case. In our series there was 1 such instance in a man 27 years of age, who underwent nephrectomy for a tuberculous kidney and died nine days later from infection of the wound, erysipelas and septicemia. The kidney removed at operation showed no glomerular disease, but the one obtained at postmortem showed acute glomerulonephritis and numerous typical embolic lesions. There was no endocarditis.

Fahr believes that embolic lesions occurring in the absence of endocarditis are caused by clumps of bacteria which lodge in the capillaries, but it is improbable that masses of bacteria in the circulating blood are of sufficient size and firmness to produce infarction. They no doubt lodge in the capillaries, but the necrosis of the glomerulus is probably a toxic effect rather than the result of infarction.

It may be concluded that embolic glomerulonephritis occurs chiefly in association with subacute streptococcic endocarditis, but it may also develop occasionally in rheumatic endocarditis, in acute

infarction of a glomerulus is seen in hypertension with acute uremia, and is caused by necrosis and sudden occlusion of the afferent arteriole. The glomerulus shows dilated capillaries with necrosis of their walls but no thrombi. There is no resemblance to embolic glomerulonephritis.

Clawson ¹¹ obtained true embolic glomerulonephritis in rabbits by intracardiac injections of finely ground agar seeded with streptococci. Inflammatory lesions of low intensity developed about the masses of bacteria but no infarction of the glomeruli resulted.

The lesions are embolic in the sense that they are caused by the lodgement of minute infected emboli in the glomerular capillaries. Toxic substances arising from the mass of bacteria are presumably responsible for capillary thrombosis and necrosis. The focal character of the lesion indicates that the bacterial bodies and not their soluble toxins are responsible. Neither Löhlein nor Fahr was able to demonstrate bacteria in the embolic lesions, but Baehr succeeded in demonstrating bacteria in 5 instances. Even after prolonged search we have been unable to find bacteria in our material, although all the cases showing large fresh lesions were investigated.

In the case of embolic lesions without endocarditis it is easily possible that bacteria taken up by polymorphonuclear leukocytes and subsequently liberated in the glomerular capillaries by necrosis of the leukocytes, may produce focal lesions. Fahr demonstrated bacteria in the lesions in his case of embolic glomerulonephritis without endocarditis.

What is the ultimate fate of the fresh hyaline lesion? Does it disintegrate and disappear, or is it converted into a fibrous lesion? Baehr is definitely committed to the latter view. He believes that fibroblasts from without invade the hyaline lesion and convert it into fibrous tissue. Fahr believes that the hyaline lesion becomes fibrous but does not indicate how the process comes about. In preparations stained with hematoxylin and eosin one gets the impression that there are transitions between the fresh hyaline and the fibrous lesions, but with the azocarmine stain it is clear that this is not the case. The hyaline lesions become necrotic, disintegrate and disappear. Around some of them there is a thickening of the basement membrane which produces a fibrous appearance, but this alteration is around the lesion and not within it.

In the subacute group there were 13 instances in which only the

Complete closure of a glomerulus results in atrophy of its associated tubule. When a large proportion of the glomeruli are obliterated by embolic lesions, death results from uremia. In 5 cases of subacute bacterial endocarditis the embolic lesions were so extensive that uremia developed. In 3 of these, large fibrous lesions were responsible and in 1, large, fresh, hyaline lesions. In the fifth case the glomeruli were destroyed by large, fresh, hyaline lesions and large, fibrous crescents which compressed the glomeruli.

In the subacute group there were 7 instances of acute glomerulonephritis and 3 of chronic glomerulonephritis in which there was ample anatomical evidence of severe renal insufficiency. Therefore, in the subacute group there were 15 cases with anatomical evidence of marked renal insufficiency, 10 due to diffuse glomerulonephritis, and 5 due to embolic lesions. Schiele¹² reported 2 cases of embolic glomerulonephritis terminating in uremia. In 1 of these there was no endocarditis. In 77 cases of subacute streptococcus endocarditis Baehr⁶ reported nine deaths from uremia, two of which were due to acute and seven to chronic glomerulonephritis.

Epithelial crescents are a prominent feature in subacute bacterial endocarditis. They were found in 31 per cent, always in association with diffuse or embolic lesions. In 3 instances they were associated with diffuse glomerulitis, in 2 with embolic lesions, and in 29 with both diffuse and embolic lesions. Shortly after its formation, fine fibers appear between the epithelial cells of the crescent (Fig. 9). These increase in size and number until the entire crescent has a dense fibrous structure (Fig. 10). The fibers are formed entirely under the influence of the epithelial cells; there is no invasion of the crescent by fibroblasts from without. After the fibers have become coarse they give the staining reactions of collagen. Fibrous crescents compress the glomeruli and stop the capillary circulation. The result of this compression is a collapse of the capillaries and a marked thickening of their basement membranes. Finally the thickened membranes fuse into a rather homogeneous mass. Many glomeruli are obliterated by epithelial crescents.

It is to be noted that in glomerulonephritis fibers form inside the capillaries from endothelial cells and in the crescents from mesenchymal epithelial cells. The term "fibroblast" can no longer be restricted to cells of the connective tissues.

fresh hyaline lesions were present. Five of these were of only six weeks duration, but in 2 the endocarditis had been present for five months, and in 1 for one year. The presence of fresh lesions only does not necessarily indicate that the disease is of short duration. In 3 instances only fibrous lesions were found. The duration of these cases was two months, ten weeks, and six months respectively. In the great majority of cases both types of lesions are present.

The Fibrous Lesions: The fibrous lesions are a little less frequent than the fresh hyaline. With azocarmine the former are colored a deep blue and the latter a bright red. The contrast between the two types is very striking. Evidently a somewhat longer time is required for the development of the fibrous lesions than for the fresh hyaline, since the former are rarely found in any form of acute endocarditis. They were numerous in one case of seven weeks duration, but usually they are not present in cases of less than three months standing. The lesions vary in size from small focal fibrotic areas to diffuse fibrosis of the entire glomerulus. In its earliest stages (Fig. 6) the fibrous lesion shows a thickening of the basement membrane of a group of capillaries. There is no increase of endothelial nuclei, no thrombosis, and no necrosis. The basement membranes increase in thickness at the expense of the capillary lumens. The fibers are all thickened basement membranes. There is no invasion of fibroblasts. When the membrane is moderately thickened it gives the staining reactions of collagenous fibers.

The large diffuse lesions (Figs. 7 and 8) are formed in the same way as the focal fibrous areas. Occasionally small, fresh, hyaline lesions are found in a diffuse fibrous glomerulus, but the fibrous lesions do not develop from the hyaline form. The thick fibers may be followed directly into normal basement membranes (Fig. 8). The end result of a fibrous lesion is a dense mass of thick membranes which occludes the capillaries completely. Endothelial and epithelial nuclei persist for a long time but finally disappear and a hyaline glomerulus results.

The focal character of the lesion suggests that it may be caused by the lodgement of bacteria in the capillaries, but bacteria have never been demonstrated. If bacteria are responsible we must believe that the injury is not so intense as in the case of the hyaline lesions. No capillary thrombi are formed and no necrosis occurs, but a type of proliferative inflammation is induced.

and may be found at any time during the course of the disease. The frequent absence of embolic lesions in typical clinical examples of subacute bacterial endocarditis has not been explained.

Diffuse glomerulitis is frequently found in association with embolic lesions.

Epithelial crescents frequently cause atrophy of the glomerular tufts by compression. Fibers form between the epithelial cells and convert the crescent into a dense fibrous structure. These fibers are of epithelial origin.

In the glomeruli, fibers which later give the staining reactions of collagen are formed from three distinct sources — intracapillary fibers from the endothelial cells, fibers formed from thickened capillary basement membranes, and fibers formed by the epithelial cells of the crescents.

SUMMARY

Two forms of glomerulitis, namely, diffuse and embolic, are found in association with endocarditis.

Diffuse glomerulitis was found with the various types of endocarditis as follows: acute rheumatic 22.2 per cent, acute primary bacterial 28.6 per cent, subacute bacterial 64.8 per cent, and secondary acute 33.3 per cent. It is characterized by an increase in the number and size of the endothelial cells and often by thickening of the capillary basement membrane. The extent of capillary obstruction is usually much less than in clinical acute glomerulonephritis, but in 7 instances of subacute endocarditis glomerulitis had reached the clinical level. Diffuse glomerulitis bears some relation to the intensity and duration of septicemia.

Embolic, or focal glomerulitis was found in the different forms of endocarditis as follows: acute rheumatic 2.9 per cent, acute primary bacterial 7.1 per cent, subacute 52.8 per cent, and secondary acute 5.8 per cent. In 1 instance there was no endocarditis.

Two distinct types of embolic lesions occur — the fresh hyaline and the fibrous.

The fresh hyaline lesion in its simplest form is a capillary thrombosis and all the smaller lesions are readily recognized as such. The larger lesions are composed of many thrombosed capillaries which may be identified until the capillary walls have undergone necrosis. The hyaline lesion is not an infarct but a thrombosis and necrosis of capillaries resulting from the lodgement of bacteria. The necrotic portion of the glomerulus disintegrates and disappears.

The fibrous lesion is a reaction characterized by a marked growth of the basement membranes of the capillaries. The thickened membranes obliterate the capillaries and give the glomerulus a fibrous structure. The fibers are formed entirely from basement membranes; there is no invasion by fibroblasts from without. The fibrous lesion, like the fresh hyaline, may involve one or more lobules or the entire glomerulus. It develops independently of the fresh hyaline lesions.

In subacute bacterial endocarditis there were 15 instances of severe renal insufficiency, of which 5 were due to embolic glomerulonephritis, 7 to acute and 3 to chronic glomerulonephritis.

The fresh, hyaline, embolic lesions develop earlier than the fibrous

DESCRIPTION OF PLATES

PLATE 106

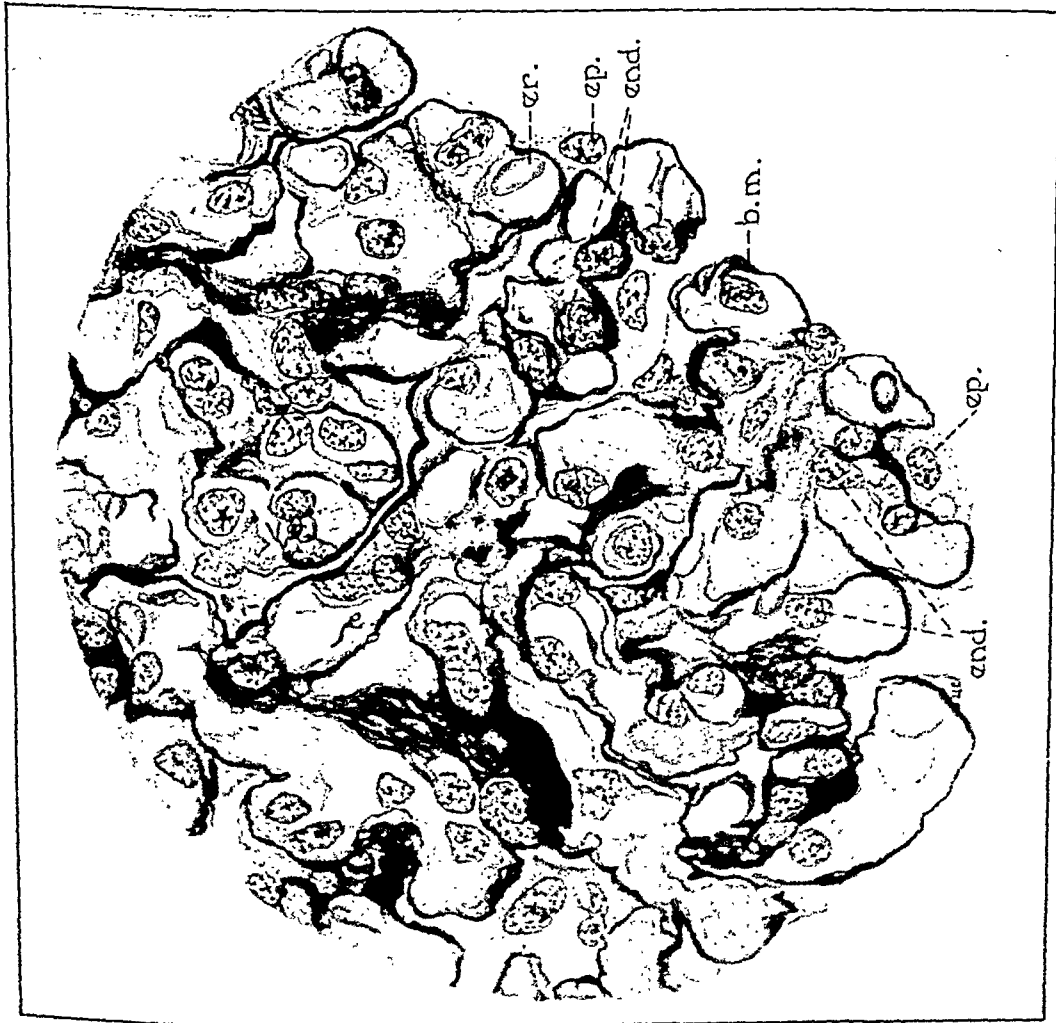
- FIG. 1. 31-1209. Diffuse glomerulitis. Part of a glomerulus from a case of subacute bacterial endocarditis. Azocarmine stain. There is a definite increase in the number and size of the endothelial cells (end.) and there is also a moderate uneven thickening of the capillary basement membrane (b. m.). The lumens of the capillaries are definitely narrowed, but the obstruction is not so prominent as in clinical acute glomerulonephritis. $\times 750$.
- FIG. 2. 27-445. Small, fresh, hyaline, embolic lesion — capillary thrombosis. Small portion of a glomerulus from a case of acute rheumatic endocarditis. Azocarmine stain. The thrombus distends the capillaries but there is no necrosis of the capillary wall. The small embolic lesions in subacute bacterial endocarditis are all of this type. Ep., epithelial cell; end., endothelial cell; b. m., capillary basement membrane; t., thrombus. $\times 750$.
- FIG. 3. 30-819. A part of a large, fresh, hyaline, embolic lesion from a case of subacute bacterial endocarditis. Azocarmine stain. The thrombotic material (t.) fills some of the capillaries completely and others partially. The capillary basement membrane (b. m.) is present in some places but absent where necrosis has occurred. Endothelial nuclei (end.) may persist after the basement membrane and epithelial cells have disintegrated. The outlines of the capillaries may usually be recognized in large, fresh, hyaline lesions. Ep., epithelial cell. $\times 750$.

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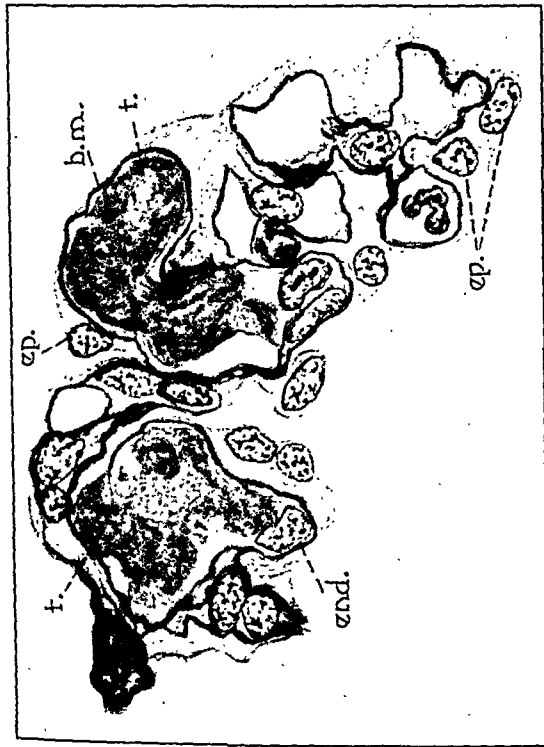
PLATE 107

- Fig. 4. Fresh, hyaline, embolic lesion. From the same case as Fig. 3. Hematoxylin-eosin stain. The individual thrombosed capillaries may be seen. $\times 400$.
- Fig. 5. Higher magnification of a small part of the lesion shown in Fig. 4. Note the nuclei of epithelial cells between the thrombosed capillaries.
- Fig. 6. 16-80. Subacute bacterial endocarditis. Area from a glomerulus showing a small fibrous lesion. Azocarmine stain. The hyaline bands (h.) are formed by thickening of the basement membranes of the capillaries. Normal basement membranes are continuous with thickened ones. The endothelial cells (end.) are increased in number and size in capillaries outside of the fibrotic area. B. m., normal capillary basement membrane; ep., epithelial cells. $\times 750$.
- Fig. 7. 30-1522. Diffuse fibrous lesion. From subacute bacterial endocarditis. Azocarmine stain. The glomerulus is largely replaced by coarse fibers. $\times 400$.

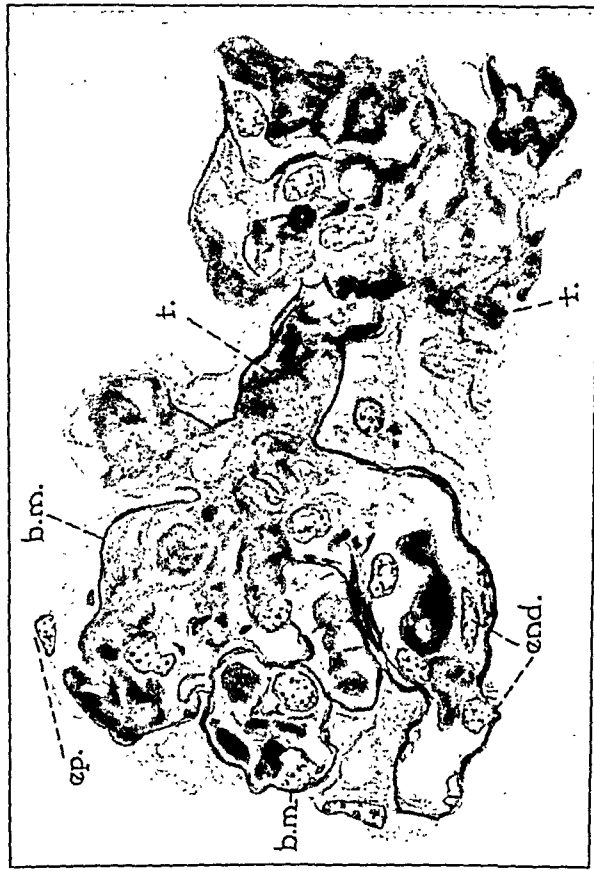


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Glomerular Lesions Associated with Endocarditis

PLATE 108

- FIG. 8. From the same case as Fig. 7. Azocarmine stain. The coarse fibers (c.f.) are continuous with definite capillary basement membranes. The majority of the nuclei are probably endothelial (end.). $\times 750$.
- FIG. 9. 16-89. Epithelial crescent from subacute bacterial endocarditis. Azocarmine stain. Small fibers (f.) are shown among the epithelial cells (ep.). C. b. m., capsular basement membrane. $\times 750$.
- FIG. 10. 31-455. Epithelial crescent from subacute bacterial endocarditis. Azocarmine stain. The epithelial cells (c.ep.) are largely replaced by coarse fibers (f.). C. b. m., capsular basement membrane. $\times 750$.

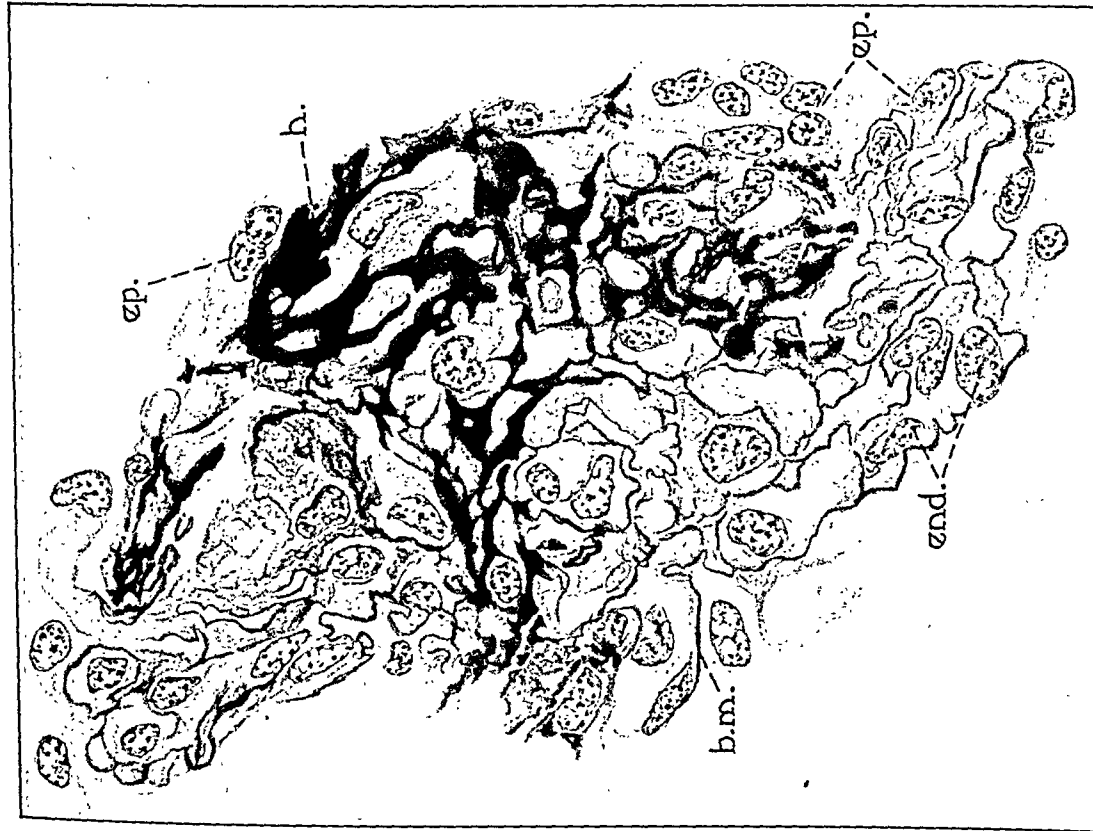


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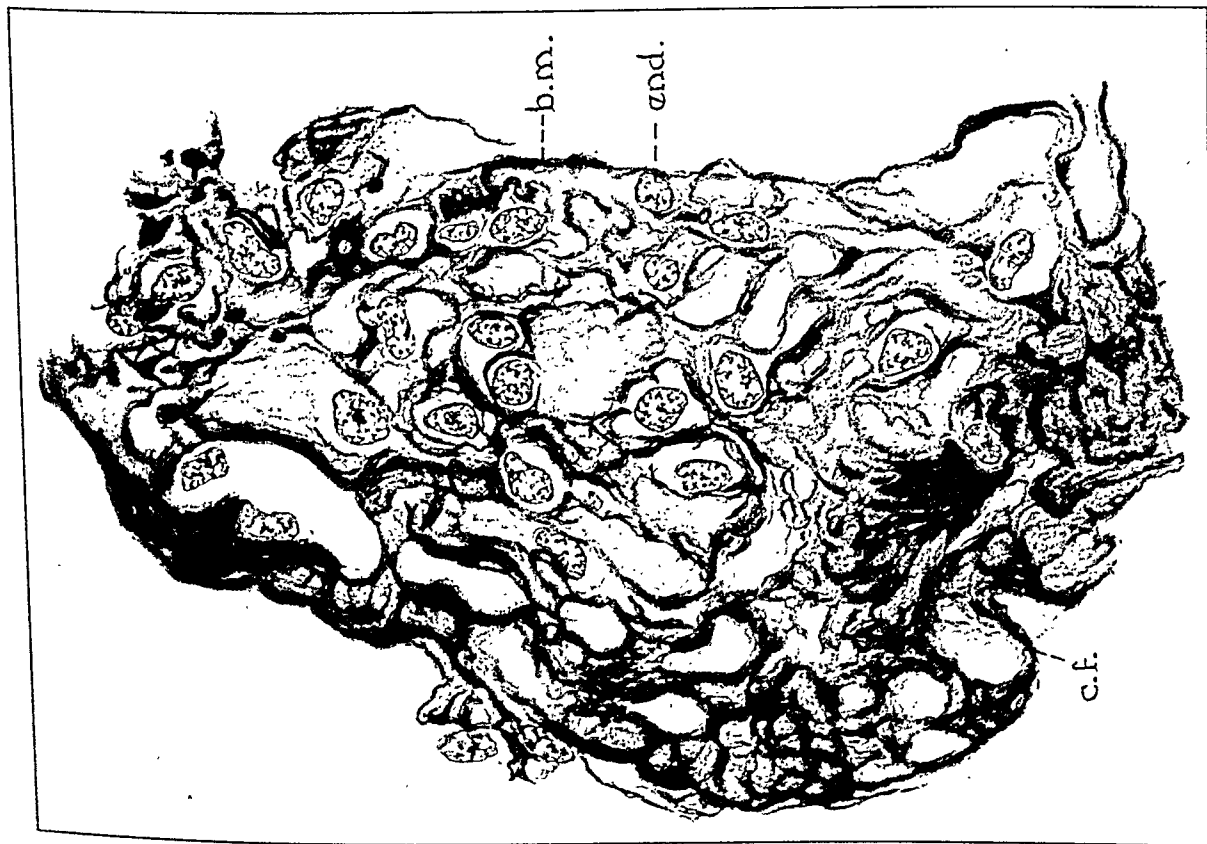
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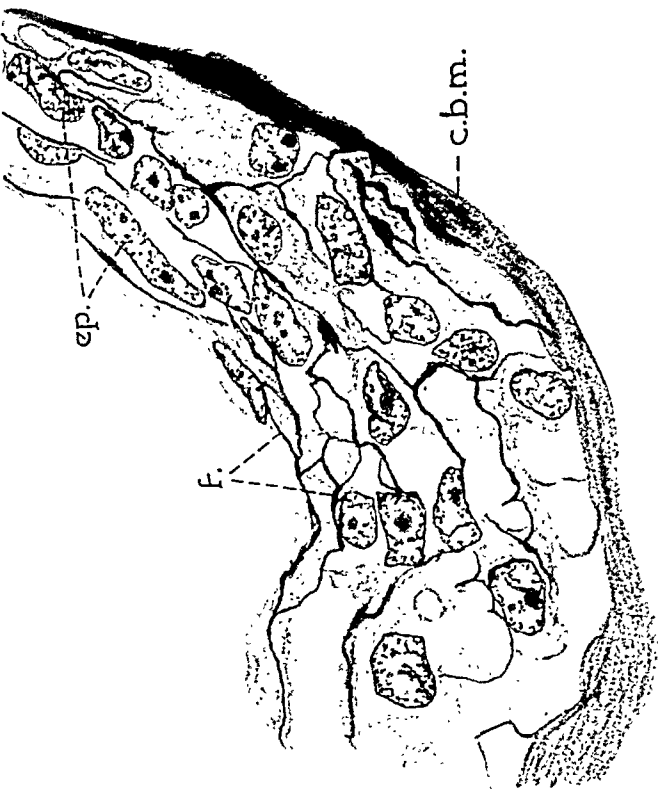
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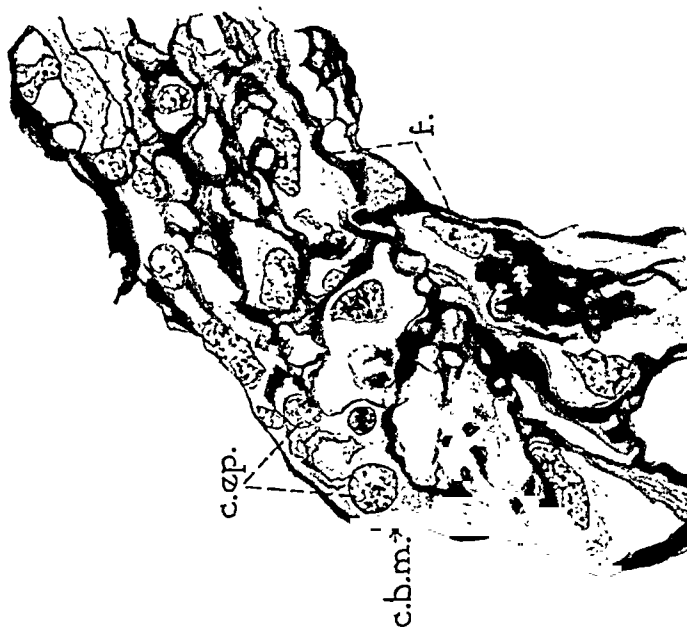
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Takayasu¹ failed to see any very marked glomerular lesions in a study of sixty rabbits poisoned with uranium nitrate, potassium bichromate and mercuric chloride, in which changes termed tubular nephritis by Schlayer and Hedinger² had been found. Christian³ described for the first time the occurrence of "hyaline droplets" within the glomerular capillary walls of rabbits receiving uranium nitrate and potassium bichromate. Christian felt the presence of such bodies must indicate that considerable injury to the capillaries of the glomeruli had been brought about by the chemicals employed. In later studies Christian, Smith and Walker,⁴ and Christian and O'Hare⁵ noted that the hyaline droplets were followed by more advanced degenerative changes consisting of hyaline thrombus formation, hemorrhage, bleeding in the glomeruli with dilatation of the capsular space, proliferation of capillary endothelium and also of the capsular epithelium. Baehr⁶ states that Mackenzie was able to demonstrate occasional hemorrhages or "blood cysts" in the glomeruli of certain rabbits injected with uranium nitrate. Suzuki⁷ also noted glomerular hemorrhage and necrosis in one uranium-damaged kidney. Scheel⁸ mentions the appearance of necrosis of the glomeruli in uranium nephritis but gives no further details. After Christian the first notable attempt to ascertain the nature of glomerular lesions induced by uranium nitrate was made by Baehr. By subcutaneous administration he obtained, in one of ten rabbits, a collapse and thickening of the glomerular capillaries in which there was a homogeneous protoplasmic substance. The capsular space contained albumin, while the loops were blended with Bowman's capsule and formed epithelial crescents. More constant results were obtained after injection of very small quantities of uranium directly into the renal artery on one side. By this technique 40 to 50 per cent of glomeruli were affected. According to Baehr the evolution of the glomerular lesions could be divided into four stages. The first was characterized by coagulation necrosis, failure of the endothelial nuclei to stain, a diminution of blood and the presence of a reticulated coagulated substance in the capillaries. The epithelial covering of the loops was often seen to be necrotic. From the damaged vessels there was an extravasation of blood or plasma into the capsular space. In some instances entire glomeruli were affected but in others the injury was limited to certain loops. The afferent arterioles were plugged with granular masses similar

GLOMERULAR CHANGES IN THE KIDNEYS OF RABBITS AND MONKEYS INDUCED BY URANIUM NITRATE, MERCURIC CHLORIDE AND POTASSIUM BICHROMATE *

WARREN C. HUNTER, M.A., M.D., AND JOE M. ROBERTS, A.B.

(From the Department of Pathology, University of Oregon Medical School, Portland, Ore.)

The injurious effects of the salts of uranium, mercury and chromium on the epithelium of the renal tubules have long been recognized. Of these substances uranium nitrate particularly has been employed as a nephrotoxin by numerous experimental workers in attempts to solve the difficult problems of renal function. All of the poisons mentioned cause definite and unmistakable lesions in the proximal convoluted tubules. Although certain cytological changes have long been noted in the glomeruli of kidneys injured by the salts of heavy metals, these alterations are histologically much less prominent than in the tubules, and possibly for this reason almost universally have been considered of less significance than the outspoken and widespread tubular necrosis, in spite of certain clinical evidence to the contrary.

The usual tissue stains show degeneration and even necrosis of both capillary and capsular glomerular epithelium, often a granular albuminous substance in the capsular space, occasional intra- and extraglomerular hemorrhages, diminution, absence or excess of blood within the glomerular capillaries during the acute stage of injury by uranium and similar substances. The histological state of the capillary basement membrane cannot well be studied by ordinary methods and has therefore generally been neglected. As the lesions of chemical nephrosis become chronic there is seen a proliferation of the capsular epithelium sometimes forming epithelial crescents, thickening of Bowman's membrane and apparent atrophy, fibrosis, or even hyalinization of the glomerular tufts.

A brief review of the literature on experimental nephritis will lend emphasis to the preceding statements.

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tion. In other instances the capsular spaces appeared dilated and cystic with apparent atrophy of the glomeruli, but even in the most markedly scarred and contracted kidneys there were always some hypertrophied, blood-filled and apparently functioning glomeruli. With mercuric chloride glomerular damage, evidenced by necrosis of epithelium, intraglomerular hemorrhages and hyaline droplet formation, was less constant than in the uranium kidneys. In a comprehensive review of acute experimental nephritis MacNider¹² in summary states that there is more histological evidence of a glomerular injury from bichloride of mercury than from either uranium or the chromates, the injury consisting of intense congestion with or without an exudate, hyaline changes in the endothelium or thrombus formation. Regarding chromates he asserts that the participation of the glomeruli in the nephritis is late.

A distinct advance in the finer histopathology of the renal glomerulus came with the discovery by McGregor^{13, 14} of the great value of Mallory's anilin blue-Heidenhain-azocarmine stain in differentiating the various glomerular elements, clearly outlining the capillary basement membrane, and thereby making it possible not only to study changes hitherto unperceived in this structure, but also to distinguish the endothelial and epithelial cells of the capillaries from each other. By means of this stain McGregor¹⁵ later demonstrated characteristic basement membrane alterations in the kidney in essential hypertension. More recently Bell,^{16, 17} employing the same staining method, has proved the existence of important alterations in the glomerular basement membrane of the kidney in lipoid nephrosis and in the toxemias of pregnancy. In each of the conditions named there is a tendency to pronounced thickening of the membrane.

MATERIALS AND METHODS EMPLOYED IN PRESENT INVESTIGATION

As far as we have been able to find in the available literature, the azocarmine stain has never been used as an aid in determining the nature of glomerular alteration in experimental lesions induced by uranium, mercury and chromium salts. Therefore, after having first studied a considerable number of renal lesions in man and satisfying ourselves that for such purposes the azocarmine stain is a

to those in the capillaries of the tufts. The second stage was marked by a swelling and proliferation of glomerular and capsular epithelium with the formation of syncytial structures and multinucleated giant cells, the two types of cells forming a network leading to obliteration of the capsular space. In the third phase the capillaries and vasa afferentia became more permeable, filled with blood, and sometimes formed large blood lacunae. The final stage was characterized by hyaline degeneration of the markedly injured loops with partial adhesion to the capsule. Baehr concluded that a glomerulonephritis could be produced artificially, in the manner described, with but slight evidence of tubular damage. The overgrowth of capsular epithelium was felt to be the result of stimulation by the exudate filtered through the glomeruli. Suzuki⁹ in 1926 published the results of another series of experiments with uranium, in which he advances the opinion that the thickening of the glomerular loops has its origin in the formation of a connective tissue layer in the glomerular walls. Collapse of the glomeruli is the result of contraction of the connective tissue growth in the walls. He further states that there is sometimes proliferation and swelling of the glomerular endothelium. Suzuki makes the interesting observation that hyaline droplet degeneration occurs only in the epithelium, never in the ingrown connective tissue.

In the course of an experimental study concerned with acquired resistance of regenerated renal tubular epithelium to the nephrotoxic effects of uranium nitrate and sublimate, Hunter^{10, 11} observed in addition to degeneration and necrosis of the glomerular and capsular epithelium of rabbits receiving uranium nitrate the quite frequent occurrence of a few erythrocytes in the subcapsular space. It was felt that these must have escaped from the capillaries largely by diapedesis, made possible by injury of the capillary endothelium. Albuminous material in the capsular space also indicated glomerular injury. Christian's observation concerning the constancy of hyaline droplets was confirmed. In the chronic stages of uranium nephritis there were invariably some alterations in the glomeruli, although the tubular lesions were always more prominent. After acute injury repair of the epithelium covering the tufts and Bowman's membrane began early and not infrequently was excessive, leading to the formation of epithelial crescents or even obliteration of the capsular space, glomerular atrophy and hyaline degeneration.

are quite large, of variable shape, depending upon their location, and poor in chromatin granules. The latter tend to assume a peripheral location within the nucleus and stain faintly with carmine. The cell cytoplasm is granular, often abundant, and stains a pale blue. Epithelial cells are rarely observed over the convexity of the peripheral loops and are most abundant in the interstices between the capillaries, where they are easily distinguished from connective tissue nuclei by size and staining characteristics and from the endothelial cells by their position external to the basement membrane. The average number per glomerulus, arrived at by counting all cells in five to ten glomeruli from the kidneys of four normal animals, was found to be 30.1 epithelial cells.

As in man the capillary endothelial cells are much less numerous, according to our calculations averaging 8.2 per glomerulus, giving an average ratio of 3.7 epithelial cells to each endothelial cell. The structure and staining reactions are quite comparable to the human kidney in that the nuclei are of variable shape (according to location), stain deeply with carmine, contain a larger number of chromatin granules than the epithelium and possess one or two prominent nucleoli. Cytoplasmic substance is rarely demonstrable. The nuclei of the endothelium lining the vasa afferentia and vasa efferentia are oval and distinctly elongated. Whenever glomeruli are cut so that the afferent or efferent vessels are visible for any appreciable distance, connective tissue cells with long, oval-shaped, heavily carmine-stained nuclei can be made out readily between the capillaries. More frequently such cells are lacking because the vessels soon break up into many branches between which connective tissue nuclei are rarely found.

The outer layer of Bowman's capsule is made up of bluish-staining fibrils which join with the stroma between the adjacent tubules, while the inner layer appears to be quite homogeneous and stains dark blue with azocarmine. In the normal rabbit the entire thickness of the membrane is not great. Covering its inner surface is a single layer of flattened epithelium with nuclei identical in appearance with those covering the capillaries. The epithelial lining is often not demonstrably a continuous one.*

* Wilbur¹² has recently reported the results of quantitative counts of the various glomerular cells in the human kidney. He finds that in the ordinary glomerulus from one-fourth to one-sixth of all nuclei, exclusive of those of leukocytes, are endothelial cells.

valuable one, we next proceeded to investigate the histology of the normal rabbit and monkey kidney. Having thus established the normal it became necessary to obtain material for the investigation of possible pathological changes in kidneys injured by chemical nephrotoxins. The late Dr. A. S. Warthin generously made available the uranium and mercury rabbit material which was studied in his laboratory by the senior author. Sections of the uranium and bichloride series stained with hematoxylin-eosin, Van Gieson's stain and Mallory's phosphotungstic acid hematoxylin had been kept and were available for review. From the large number of kidneys seventy-two were selected and stained with azocarmine.

HISTOLOGY OF THE NORMAL RABBIT GLOMERULUS

The structure is essentially the same as McGregor¹³ has described for the human glomerulus, but exhibits certain minor differences which will have to be mentioned. The rabbit glomerulus is appreciably smaller than that of man, a factor which makes it more difficult to study. In most instances the afferent arteriole apparently passes only a short distance into the capsular space before breaking up into numerous capillaries. Commonly one sees most of the capillaries cut either in cross-section or tangentially (Fig. 1). The basement membrane is of uniform thickness except at points of branching of the capillaries where it widens out and becomes slightly irregular. In the normal state it stains deeply and evenly with the anilin blue component of the azocarmine stain. External to the membrane there is often visible a thin and slightly ragged granular substance, at times having a demonstrable connection with the cytoplasm of the capillary epithelium. On changing the focal plane of the oil immersion objective the membrane sometimes appears to consist of two layers, but often remains a single solid line. At the periphery of the glomerulus, where the loops are not so closely approximated as deeper within the tuft, the outline of individual loops can be followed with certainty. Using this method of examination it is found that the capillary walls exhibit comparatively little secondary undulation, thus differing from the human glomerulus in which secondary foldings are very common.

The glomerular epithelium, *i. e.*, the epithelium covering the capillaries, bears a strong resemblance to that in man. The nuclei

THE GLOMERULAR CHANGES PRODUCED BY URANIUM NITRATE

The effect of this poison upon the glomeruli, as well as upon the convoluted tubules, varies somewhat according to the acuteness or chronicity of the nephropathy. Accordingly we have divided the kidneys into two groups, classing as acute those from eighteen rabbits which died or were killed between three and twenty-eight days, following one or at most two subcutaneous or intravenous injections of uranium nitrate (Table II). The chronic series includes seventeen animals receiving increasingly larger doses over a period of months and exhibiting definite chronic renal lesions, both grossly and microscopically.

In all of the kidneys of the acute group there are varying degrees of necrosis of the epithelium lining the proximal convoluted tubules, the extent and degree depending upon the size of the dose received. In the presence of visible tubular necrosis it is not unreasonable to expect alterations in the glomeruli as well, since the noxious agent must first have passed through them. It was hoped that the azocarmine stain would bring out something more than the well known effects upon the capillary endothelium and epithelium.

As shown in Table II all of the changes demonstrable with ordinary stains were recognized in azocarmine preparations as well. But in addition to these (obvious cellular necrosis, swelling and desquamation of epithelium, a general decrease of blood in the capillaries, frequent occurrence of a finely granular material in the capsular space, intraglomerular hemorrhages and an apparently normal Bowman's capsule), we have found other morphological disturbances apparently hitherto undescribed, particularly in the capillary basement membrane. A more detailed account of this lesion will be given later.

As one phase of the problem it appeared worth while to count the actual number of glomerular and capsular cells. Such a task is tedious and unless the capillaries contain blood enough to distend them partially one cannot always distinguish epithelium and endothelium, even though the nuclei stain differently. One must also depend upon the relation of individual cells to the basement membrane as a means of identification. In order to minimize the chances of error we began with a study of normal rabbit and monkey kidneys

THE NORMAL MONKEY GLOMERULUS

Aside from its smaller size and consequently fewer number of all types of cells, the renal glomerulus of *Macacus rhesus* monkeys differs in no important respects from that of the human as described by McGregor.¹³ A minor variation occurs in Bowman's membrane which in the monkey tends to be more wavy than in man. We have found the average number of glomerular endothelial cells per unit structure to be 10.2, with an average of 40 glomerular epithelial cells, giving a ratio of 3.9 epithelial cells to one endothelial cell. The average figure obtained for capsular epithelium was 7.9 (Table I).

TABLE I

Comparison of Glomerular Cells in Normal and Nephropathic Rabbit Kidneys

Normal		Uranium nitrate		Mercuric chloride		Potassium bichromate
		Acute	Chronic	Acute	Chronic	Acute
Average number glomerular endothelial cells . .	8.2	5.7	8.2	3.6	5.8	6.9
Average number glomerular epithelial cells . . .	30.1	23.4	28.1	16.1	30.7	27.3
Average ratio of endothelial to epithelial cells	3.7	4.1	3.6	4.4	5.2	3.9
Average number capsular epithelial cells	7.1	4.7	9.6	5.1	8.7	5.8

Comparison of Glomerular Cells in Normal and Nephropathic Monkey Kidneys

Normal		Acute potassium bichromate kidney	Chronic potassium bichromate kidney
Average number glomerular endothelial cells	10.2	8.0	14.6
Average number glomerular epithelial cells	40.0	34.2	57.7
Average ratio of endothelial to epithelial cells	3.9	4.3	3.9
Average number capsular epithelial cells	7.9	9.8	10.8

TABLE II
Acute Effects of Uranium Nitrate on Renal Glomeruli of Rabbits

No.	Total dosage	Duration of experiment, days	How administered	Average % glomerular endothelial cells	Average % glomerular epithelial cells	Average ratio of endothelial cells to epithelial cells	Average % of capsular epithelial cells	Changes in glomerular basement membrane	Rowman's membrane	Intrglomerular hemorrhage	Glomerular blood content	"Droplet degeneration"
RFI	0.0005	4	Intravenous	5.1	20.5	4.0	4.2	Frayed, stains poorly	Negative	Few	Slight	Few
R-85 A	0.001	4	Subcutaneous	5.8	23.3	4.0	5.8	Poorly stained, frayed?	"	Many	Slight	Moderate
R-5-I	0.001	3	Intravenous	6.3	26.5	4.2	6.6	Negative	"	None	Moderate	Numerous
R-6-I	0.001	3	Intravenous	5.5	22.5	4.1	4.1	Split into layers, fragmented	"	Many 8.4%	Moderate	Numerous
BKI	0.0015	17	Intravenous	8.2	34.6	4.3	4.2	Slightly beaded and fuzzy	"	None	Marked	Few
BOI	0.0015	17	Intravenous	8.6	28.4	3.4	5.0	Slight splitting, stains well	"	Few	Marked	Numerous
BRI	0.0015	18	Intravenous	6.2	22.6	3.7	3.6	Frayed, beaded, stains poorly	"	Many	Slight	None
R-1-IV	0.002	3	Subcutaneous	4.2	19.0	4.4	7.0	Frayed in glomeruli with hemorrhage	"	Many	Slight	Few
R-2-IV	0.002	3	Subcutaneous	5.9	29.5	5.1	6.7	Frayed, split when hemorrhage present	"	Many	Moderate	Numerous
R-10 A	0.003	16	Subcutaneous	6.8	26.3	3.4	6.6	Negative	"	None	Marked	None
R-7 A	0.003	17	Subcutaneous	4.2	19.4	4.8	4.7	Split, fragmented, beaded	"	None	Moderate	Few
R-16 A	0.009	28	Subcutaneous	8.0	30.8	3.9	5.0	Beadings, splitting and rupture	"	Few	Marked	None
R-17 A	0.009	25	Subcutaneous	6.4	26.2	4.0	2.0	Beadings, splitting and rupture	"	Many 4.9%	Marked	Few
R-76 A	0.009	24	Subcutaneous	4.8	19.8	4.1	1.8	Faintly stained, thinner than normal	"	Few	Slight	None
R-77 A	0.009	26	Subcutaneous	5.0	19.6	3.8	2.6	Stains lightly and unevenly	"	Few 1.1 %	Slight	Few
R-79 A	0.009	24	Subcutaneous	3.4	15.0	4.5	4.6	Frayed and stains poorly	"	Few 2.0 %	Slight	Few
R-1 0.009	0.009	3	Subcutaneous	3.6	18.0	5.1	5.2	Beaded, unevenly stained, frayed	"	None	Slight	Numerous
R-2 0.009	0.009	6	Subcutaneous	5.0	20.5	4.2	6.6	Beaded, unevenly stained, frayed	"	Few	Slight	Numerous

and did not attempt counts on pathological glomeruli until reasonably certain of success. Experience showed that if the counts were made on glomeruli having patent or blood-filled loops not more than five to ten need be counted in each animal. When only a part of a glomerulus was included in the section it was found necessary to dismiss it from consideration, even if the loops were patent, because the small number of cells often gave false ratios. Using the average ratio between endothelial and epithelial cells as a basis it was found unnecessary to measure the diameters of individual glomeruli since the dimensions naturally vary with the blood content and patency of the loops, as well as the point where it happens to be cut in sectioning. The constancy of the figures shown in Table II attests the soundness of this basis of computation. In the majority of acute uranium kidneys there is a decrease of approximately one-third in the number of glomerular epithelial and endothelial cells, leaving the ratio between them practically unchanged (Tables I and II). The diminution of capsular cells is subject to greater variability, accounted for in part by the differences in the size of the glomeruli examined.

The hyaline droplets described by Christian as occurring in the capillary walls are present in fourteen of the eighteen kidneys of the acute series. In sections stained with azocarmine these bodies are of different sizes but never of great bulk, and have a reddish or orange color, depending upon the focal plane of the oil immersion objective. Often they are seen to lie in the capillary walls but in our experience, as well as that of Suzuki, appear constantly within the cytoplasm of glomerular epithelial cells (Fig. 2). At times the location seems to be intranuclear as well. We have also observed the droplets within the lumina of the capillaries but, like Christian, are unable to state whether or not such a position is due to artefact.

Azocarmine stains all connective tissues and collagenous substances a deep and dark blue, a property of great importance in the study of the glomerular basement membrane which apparently is collagenous in nature. In the acute group there are with one exception varying degrees of alteration in the basement membrane. Most frequently the homogeneous membrane substance is split up into fine fibrils and these in turn are oftentimes either fragmented or give the impression of beading with alternate light and dark staining. The picture may be best compared to the appearance of a frayed

cotton string (Fig. 3). In a few cases splitting and beading are absent, the membrane simply failing to stain well and appearing fuzzy. In these kidneys the intertubular connective tissue and Bowman's membrane stain well, indicating that the abnormal staining of the capillary basement membrane is not due to faulty technique. With the picture of the normal glomerulus constantly in mind we endeavored to avoid terming as pathological the fine, bluish, granular substance often visible over the external surface of the membrane and the slight irregularities in thickness and contour at points of capillary branching seen in entirely normal glomeruli. Still another source of error to be avoided was that of mistaking closely approximated basement membranes belonging to different loops for splitting or fraying.

During the acute stage the basement membrane does not give the impression of being appreciably thicker than normal. A single dose of uranium does not affect all glomeruli and those situated in the inner half of the cortex are regularly injured more than the tufts near the capsule. If this point is not kept in mind studies of the acute uranium kidney are apt to be misleading. Azocarmine also brings out the details of the intraglomerular hemorrhages so frequently seen in acute uranium poisoning. Often these occupy all except one or two loops and under low power magnification look like blood cysts. Examination under oil immersion reveals constant fragmentation and splitting of the basement membrane at the periphery of the hemorrhage, although one cannot always be certain that only one membrane is included (Fig. 3). Within the hemorrhage remnants of basement membrane enmeshed in fibrin and red blood cells are not infrequently seen. Since the hemorrhages occur almost as frequently following subcutaneous injections as after intravenous administration one can hardly escape the conviction that bleeding into the glomerular substance is an expression of injury to the capillary walls.

Repetition and increase of dosage of uranium nitrate will in time produce a striking pathological picture in the rabbit kidney. As previously described by Hunter¹⁰ practically all of the original epithelium lining the proximal convoluted tubules is in time destroyed and replaced by flattened atypical cells which are resistant to the nephrotoxin. There is a stimulation of the interstitial connective tissue and finally a contracted, granular-surfaced kidney is

Chronic Effects of Uranium Nitrate on Renal Glomeruli of Rabbits

R-9 A	0.303	157	Subcutaneous	—	—	—	—	—	Thickened 1 to 4 times, wrinkled, frayed and split	Markedly thickened	None	Moderate	Slight
R-5 A	0.223	128	Subcutaneous	—	—	—	—	—	Thickened 1 to 5 times, wrinkled and distinctly beaded	Moderate thickening	None	Slight	None
BGI	0.176	227	Intravenous	11.2	45.0	4.0	14.4	—	Thickened, split, beaded	Moderate	None	Slight	Few
R-15 A	0.136	127	Subcutaneous	16.6	18.6	1.1	15.0	—	Markedly beaded, wrinkled, slight splitting	Moderate	None	Slight	Few
BAI	0.1275	149	Intravenous	8.5	35.8	4.2	12.2	—	Thickened 2 to 3 times, fragmented, beaded and frayed	Marked	None	Slight	None
BLI	0.1275	162	Intravenous	7.0	28.2	4.0	7.4	—	Markedly frayed, some beading and thickening	Moderate	Few	Slight	None
R-12 A	0.127	115	Subcutaneous	6.0	22.1	3.6	10.6	—	Frayed and split, sometimes thickened	Marked	Few	Marked	Few
R-11 A	0.127	114	Subcutaneous	4.2	27.8	6.6	5.8	—	Thickening, fraying, beading	Marked	None	Moderate	Many
R-6 A	0.127	120	Subcutaneous	4.8	19.8	4.1	7.0	—	Beaded, split and wrinkled	Slight	None	Moderate	Numerous
BQI	0.0875	148	Intravenous	11.2	35.0	3.1	11.7	—	Split into layers, at times thickened	Marked	None	Moderate	Moderate
BXI	0.041	109	Intravenous	10.4	32.6	3.1	6.0	—	Marked fraying, beading and irregular thickening	Slight	None	Moderate	None
BTI	0.041	108	Intravenous	8.6	30.6	3.5	7.6	—	Split and beaded	Moderate	None	Slight	Slight
BJI	0.0035	91	Intravenous	8.2	24.2	2.9	11.2	—	Split, thickened, beaded	Moderate	None	Moderate	Few
R-84 A	0.121	88	Subcutaneous	8.0	27.4	3.4	14.8	—	Slight splitting and beading	Slight	Few	Moderate	Slight
R-83 A	0.121	88	Subcutaneous	7.1	24.8	3.4	8.5	—	Slight splitting and beading	Slight	Few	Moderate	Moderate
R-14 A	0.119	63	Subcutaneous	6.3	32.0	5.0	2.6	—	Frayed and split	Moderate	None	Slight	None
R-8 A	0.015	61	Subcutaneous	6.0	18.2	3.0	9.6	—	Frayed and beaded	Markedly thickened	None	Moderate	Few

Hematoxylin-eosin and Van Gieson's stains reveal these bodies to be quite like the lymphocytes of the blood in larger vessels. Their presence in the glomerular capillaries is apparently of no pathological significance. In the absence of definite knowledge of the nature of these apparent nuclei it was considered inadvisable to include them in the counts of glomerular cells.

THE NEPHROPATHY CAUSED BY MERCURIC CHLORIDE

In a previous paper Hunter¹¹ pointed out that the local corrosive action and irregularity of absorption after subcutaneous administration, the danger of thrombosis following intravenous injection and the excretion of mercury by the intestine, resulting in severe enterocolitis in itself often sufficient to kill the animal, makes sublimate an undesirable substance for the study of experimental renal lesions. It was found that the amount of mercuric chloride absorbed varied greatly, so that in certain cases several injections might be given without any very evident damage to the renal epithelium, while in other animals a like quantity provoked well marked chronic glomerulotubular lesions very similar to those produced by uranium. Glomerular injury, as evidenced by necrosis of epithelium, hemorrhages and hyaline droplet degeneration, was less frequent than in the kidneys of the uranium series.

The kidneys from many of the same animals used in the first experiments were employed in the present investigation. Of the fifteen selected for restudy four were classed as acute and eleven as chronic on the basis of the number of injections, the duration of the experiment and the pathological state of the kidney observed microscopically.

In each of the four acute cases the glomerular basement membrane exhibits degenerative changes identical with those already described for the acute uranium kidney, namely, splitting, fragmentation, beading and rupture. These phenomena are especially pronounced in R-2 0.015 (Fig. 6). In addition to extreme fraying of the glomerular basement membrane there is in this kidney marked diminution of both endothelial and epithelial cells, leaving the loops practically stripped of cells, the necrotic remains of which fill the capsular space. It is interesting to note that in spite of the very evident injury the capillaries are distended with unclotted blood. The three

found at autopsy. In the chronic stage there are obvious glomerular alterations, such as apparent atrophy, decrease in blood content of the capillaries, dilatation of the capsular space with or without granular material in it, rarely intra- and extraglomerular hemorrhage, hyperplasia of the capsular epithelium with epithelial crescent formation, obvious thickening of Bowman's membrane with epithelial cell inclusions and hyaline droplet degeneration.

The kidneys of all of the seventeen rabbits in the chronic uranium group display more convincingly the same changes in the glomerular basement membrane as in the acute stage, with the additional finding of definite thickening and not infrequently wrinkling of the membrane. The latter is not surprising in view of the shrinkage of glomeruli (Fig. 5). In the two rabbits showing the greatest thickening (R-5 A and BAI) reddish, oval-shaped nuclei are buried within the heavy membrane. Their position, external to the wavy condensed portion of the basement membrane, points to an epithelial or connective tissue origin. It is significant that in each of the two rabbits mentioned the glomerular capillaries are distended with blood in spite of the great thickening of their walls (Fig. 5). Hyaline droplets occur less frequently and in lesser numbers than in the acute phase, and again appear to lie both in the substance of the epithelial cells and in the basement membrane. Cell counts show that both endothelial and epithelial cells possess sufficient regenerative capacity to come back to approximately normal after having been reduced by about one-third during the acute stages of the disease. We are unable to discover any evidences of hyperplasia of the connective tissue cells accompanying the arterioles, and are of the opinion that such cells have nothing to do with collapse and atrophy of the glomeruli. Our observations lead us to believe that the small size of apparently atrophic tufts is due largely to collapse of the capillaries. Practically all nuclei remaining in such glomeruli have the appearance and staining characteristics of epithelium. The ability of glomerular epithelium and endothelium to regenerate in the presence of repeated damage by uranium suggests that these cells, as well as those lining the tubules, become somewhat tolerant to the drug. In addition to glomerular epithelial and endothelial cells both acute and chronic uranium kidneys frequently display intracapillary nuclei more heavily impregnated with carmine than endothelial cells and devoid of chromatin granules or nucleoli.

TABLE III
Acute Effects of Mercuric Chloride on Renal Glomeruli of Rabbits

No.	Total dose	Dose per day	How administered	Average No. glomeruli per capsule	Average No. glomeruli per capsule	Average ratio of endothelial to epithelial cells	Changes in glomerular basement membrane	Bowman's membrane	Intra-glomerular hemorrhage	Glomerular blood content	"Droplet degeneration"
R-2 0.015	0.026	3	Subcutaneous	1.8	6.2	3.7	Extreme fragmentation, splitting and rupture	Negative	Few	Marked	None
R-1 0.020	0.030	3	Subcutaneous	3.2	17.8	5.5	Fragmented, beaded, split	"	None	Slight	None
R-1 0.003 S	0.0036	8	Subcutaneous	4.6	22.5	4.9	Splitting and fragmentation	"	None	Moderate	Few
R-1 0.004 S	0.012	64	Subcutaneous	4.8	18.2	3.7	Fuzzy and beaded	"	None	Moderate	Few

Chronic Effects of Mercuric Chloride on Renal Glomeruli of Rabbits

R-1 0.006 S	0.025	29	Subcutaneous	8.1	36.1	4.6	Fuzzy and beaded	Slightly thickened	Few	Slight	Moderate	
R-2 0.003 S	0.0298	32	Subcutaneous	4.6	19.8	4.3	Thickened and beaded	"	None	Slight	None	
R-2 0.002 I	0.012	32	Intravenous	6.0	32.8	5.5	Splitting	Moderately thickened	None	Slight	Numerous	
R-2 0.005 S	0.018	35	Subcutaneous	7.2	35.0	4.8	Fuzzy, split and beaded	"	None	Slight	Moderate	
R-4 Int. I	0.021	43	Intravenous	4.6	23.8	5.2	Fuzzy and indefinite, beaded and fragmented	Slightly thickened	None	Slight	None	
R-2 0.009	0.0498	44	Intravenous	6.8	40.6	5.9	No apparent changes	"	None	Slight	Numerous	
R-1 0.007 S	0.0258	44	Subcutaneous	6.0	31.8	5.5	Fuzzy	"	Few	Moderate	Moderate	
R-2 Sub. II	0.0755	66	Subcutaneous	6.4	26.6	4.2	Trayed	Negative	None	Moderate	None	
R-1 0.001 I	0.0270	82	Intravenous	6.6	42.8	6.5	Slight splitting and beading	Markedly thickened	None	Slight	Few	
R-2 Int. I	0.0428	84	Intravenous	4.5	25.8	5.9	Wrinkling, slight beading	Slightly thickened	None	Slight	None	
R-1 Sub. I	0.1128	87	Intravenous	4.0	22.6	5.7	Uneven, split, fragmented	Negative	None	Moderate	None	

remaining kidneys of this group likewise display a significant decrease in glomerular cells, together with varying degrees of injury to the basement membrane. In contrast to the high incidence of intraglomerular hemorrhages and droplet degeneration of the capillary epithelium in acute uranium kidneys, hemorrhage occurs in but one instance of acute mercuric nephropathy, while droplets are found in two of the four animals.

In the ten rabbits receiving repeated doses of mercury there is some degree of splitting, beading or alteration in the staining quality of the basement membrane in all but one. Thickening is much less frequent than in uranium nephropathy. In the chronic mercuric chloride kidney the endothelial cells increase from an average of 3.6 in the acute stage to 5.8 per glomerulus. The glomerular epithelium, after falling to half the normal number in the acute phase, regenerates to such an extent that at the end the normal is re-established and in several instances is actually increased (Table III). As for other pathological findings there is often but not always a slight to moderate thickening of Bowman's capsule, fairly constant hyaline droplet degeneration, decrease in capillary blood content, rarely hemorrhages in the glomerular substance and the same heavily stained lymphocytic nuclei within the capillaries present in uranium nephritis.

EXPERIMENTS WITH POTASSIUM BICHROMATE

Six rabbits and six monkeys (*Macacus rhesus*) were employed in these experiments (Table IV). Of the six rabbits four had but one dose of poison and died within one to eight days. The two remaining animals first received 1 cc. of a 2 per cent solution followed by three doses of 1.5 cc. each over a period of thirty-eight days. In none of the six did the bichromate cause more than acute degeneration and necrosis of the tubular epithelium. Study of the glomeruli in azocarmine preparations reveals changes quite like those in the acute uranium and bichloride lesions, with the exception of R-6 which died on the first day of the experiment. Intraglomerular bleeding and droplet formation occurs in only two instances.

In the monkey the results are quite similar except for M-5 and M-6 (Table IV), in whom definite chronic tubular lesions were produced. In both animals the glomerular endothelial and epithelial cells show a significant increase over the normal, at the

same time preserving approximately the normal ratio (Table I). Although definitely damaged, the degenerative splitting, fragmentation and beading is less marked than in either the uranium or mercury nephropathies of rabbits.

A detailed description of the tubular involvement will be published as a separate paper.¹⁹ It is sufficient to say here that bichromate affects the epithelium of both proximal and distal convoluted tubules and that the regenerated cells are resistant to the poison.

DISCUSSION

From the standpoint of histopathology there is no doubt as to the most prominent seat of renal damage caused by the chemical substances employed in the preceding experiments. Unquestionably the tubular epithelium, particularly that of the proximal convoluted units, suffers severely and after having become necrotic and detached presents a striking picture of devastation. It is not surprising, therefore, that in the presence of clearly demonstrable tubular necrosis the comparatively slight and less easily proved glomerular changes have been regarded as relatively unimportant in accounting for the altered renal function brought about by certain chemical nephrotoxins. Perhaps this is in part a heritage handed down by the earlier experimentalists, who grouped nephrotoxic substances into two classes: (*a*) including cantharidin, arsenic and diphtheria toxin, was thought to produce deleterious effects by injury of the vascular apparatus of the kidney; while (*b*) other chemicals like uranium, sublimate and chromates, were regarded as tubular poisons.

In the foregoing pages we have attempted to gather from the literature the more important observations of glomerular injury evoked by uranium, bichloride and bichromate. There is general agreement that the changes in the glomeruli are purely degenerative during the acute stages of the process and that later both degenerative and proliferative phenomena may occur. Beyond this point there have been comparatively few attempts to analyze closely the state of the glomeruli after damaging the kidney with poisonous chemicals.

Recent years have brought significant advances in the physiology of renal function, and the modern theory of glomerular filtra-

TABLE IV

Effects of Potassium Bichromate on Renal Glomeruli of Monkeys

No.	Total dosage	Duration of experiment	How administered	Average No. glomerular endothelial cells	Average No. epithelial cells	Average ratio of endothelial to epithelial cells	Average No. capsular epithelial cells	Changes in glomerular basement membrane	Bowman's membrane	Intraglomerular hemorrhage	Glomerular blood content	"Droplet degeneration"
M-1	gm. 0.726	days 1	Subcutaneous	7.0	26.2	3.8	6.2	Stains poorly	Negative	None	Moderate	Numerous
M-2	0.200	1	Subcutaneous	6.8	30.2	4.6	6.0	Stains poorly, frayed, split, slightly beaded	"	None	Marked	Numerous
M-3	0.280	71	Subcutaneous	7.4	34.8	4.6	16.4	No apparent change	Slightly thickened	None	Moderate	Very few
M-4	0.060	22	Subcutaneous	10.8	45.8	4.3	10.6	Frayed and slightly beaded	"	None	Moderate	None
M-5	0.320	160	Subcutaneous	16.8	63.8	3.8	13.0	Frayed and beaded, wrinkled, thickened 2 to 3 times	"	None	Slight	Numerous
M-6	0.320	163	Subcutaneous	12.4	51.6	4.1	8.6	Thickening, beading, fraying	"	None	Slight	Numerous

Effects of Potassium Bichromate on Renal Glomeruli of Rabbits

R-6	0.040	1	Subcutaneous	9.5	33.5	3.5	6.0	No changes in basement membrane	Negative	None	Slight	Few
R-4	0.040	3	Subcutaneous	7.2	24.0	3.3	5.7	Slight splitting	"	None	Moderate	Numerous
R-2	0.040	5	Subcutaneous	6.0	29.0	5.1	6.7	Stains poorly, splitting and fragmentation	"	None	Moderate	Few
R-9	0.040	8	Subcutaneous	9.6	39.0	4.1	8.5	Split and beaded	"	Few	Moderate	None
R-19	0.110	38	Subcutaneous	4.6	16.4	3.5	3.6	Slight fraying, fragmentation	"	None	Slight	None
R-18	0.110	38	Subcutaneous	5.0	22.0	4.4	4.4	Slight beading and splitting	"	None	Moderate	None

materials in the urine are reduced or disappear. Certain of the chronic animals develop a polyuria.

For information regarding the blood chemistry in chemical nephropathies we turn to the literature. MacNider²⁰ has demonstrated repeatedly the occurrence of decreased phenolsulphonethalein excretion, retention of blood urea, non-protein nitrogen, creatinin and lowered alkali reserve in dogs suffering from acute or chronic uranium nephritis. In certain of the chronic cases he noted structural changes of a chronic degenerative nature in the glomeruli, — fibrosis, obliteration of some capillary loops by connective tissue — sometimes leading to hyalinization, while other glomeruli showed partial obliteration of the loops with thickened walls and blood within the lumina.

The striking similarity of the disturbances in the blood and urine in acute and chronic clinical glomerulonephritis and sublimate nephrosis in the human, and acute and chronic uranium and bichloride nephropathy of experimental animals, has not been properly appreciated. The urinary manifestations have been ascribed mainly to tubular involvement. In view of our results we find it impossible to subscribe to this view. It is not unreasonable to expect abnormal urinary function in the presence of a structural alteration of the glomerular basement membrane and covering epithelium. In this connection it is interesting to note that while the regenerated tubular epithelium becomes resistant to the particular substance employed, there are at all stages degenerative phenomena in the glomeruli.

The concept of injury to the glomerular basement membrane, with resultant altered permeability as the factor chiefly responsible for the disturbances in renal function following administration of metallic salts, is in keeping with the modern theory of renal function. If the physiologists are correct, destruction of tubular epithelium should be followed by excretion of a large amount of urine of very low specific gravity. As everyone knows quite the contrary is found. Not only is there oliguria and concentration of the urine but a retention of nitrogenous products in the blood as well. Except for the absence of edema and uremia the picture is quite similar to acute and subacute human glomerulonephritis. This concept is supported by the work of Oliver and Shevsky,²¹ who noted in one of their frog experiments a repression of urine associated with a good flow of blood through the glomerular capillaries, interpreted as possible

tion-tubular reabsorption with certain modifications is now generally accepted. More recently still the notable contributions of McGregor^{13, 14, 15} and Bell^{16, 17} have aided materially in explaining the nature and significance of glomerular lesions in human renal diseases. It now appears that the state of the glomeruli is of much greater importance than the condition of the tubules in the pathological physiology of the kidney, and that changes in the latter are largely secondary to and dependent upon glomerular disease.

The purpose of the present study was to determine with the aid of newer staining methods all of the abnormalities in glomeruli made nephropathic by metallic salts. Besides confirming the findings of many other writers recorded in the literature, we have discovered another which we believe to be quite significant, namely, the nearly constant occurrence of demonstrable alterations in the glomerular basement membrane, manifested by a splitting, fraying, fragmentation and abnormal staining reaction in azocarmine preparations. Since the glomerulus is the first structure in the kidney with which circulating poisons come into contact, it is not surprising that some of its elements may be damaged during the filtration process by which urine is formed. Such injuries may be marked, as in glomerulonephritis, or of less apparent significance, such as the thickening of the basement membrane seen in essential hypertension, lipid nephrosis and the toxemias of pregnancy. Glomerular injury may likewise occur when the substance brought to the kidney is a simple chemical poison. The lack of histological evidence of lesions is not proof that the glomeruli escape unscathed. Nor does the extensive tubular necrosis explain all of the clinical symptoms, as we shall point out presently.

Our investigation is limited to morphological glomerular alterations and we are not in a position to record from personal observations more than a few already well known facts concerning the effects of the poisons used in our experiments upon the volume output and constituents of urine. We have observed that while a transitory polyuria may follow a lethal dose of uranium or mercuric chloride, oliguria or even anuria soon ensue and may persist until death of the animal. The urine excreted is concentrated and for some days contains quantities of albumin, casts, and frequently small numbers of red blood cells. If the renal lesion becomes chronic the abnormal

increased, but this is purely a secondary feature. Our results show that the glomerular epithelium and endothelium regenerate after the initial injury, but the total is usually not greater than in normal controls. Proliferation of capsular epithelium occurs regularly and may just as properly be regarded as evidence of proliferative inflammation as it is in human glomerulonephritis. If by nephrosis one refers to that group of renal diseases predominantly degenerative in character, this designation would more nearly fit pathologically the lesions provoked by heavy metals than any other. Clinically, however, the blood and urine manifestations do not fit those of typical nephrosis and we are left without any suitable name for chemically produced renal diseases other than chemical nephropathies.

SUMMARY

1. By a special staining method (azocarmine) distinct changes, interpreted as evidences of injury, can be demonstrated in the glomerular basement membrane of kidneys damaged by uranium nitrate, mercuric chloride and potassium bichromate.

2. The lesions in the basement membrane are purely degenerative in character, present in both acute and chronic stages of chemical nephropathies, and appear to be permanent. The renal glomerulus is more vulnerable to poisons than the tubules and fails to develop the same degree of increased resistance toward them.

3. The opinion is advanced that alterations in the basement membrane of the type described may play an important rôle in the renal disturbances induced by certain metallic salts.

4. Fibrosis and connective tissue hyperplasia are not responsible for the appearance of the glomeruli in chronic uranium and sublimate nephropathies.

5. The existence of other long recognized glomerular changes demonstrable with ordinary stains is affirmed.

6. Uranium nitrate and mercuric chloride produce more histological alterations in the glomeruli than potassium bichromate.

evidence of increased density of the basement membrane. Richards²² has observed an active glomerular circulation in the absence of urine formation in the kidney of a living frog poisoned with mercuric chloride. He explains the anuria as due to increased absorption of water by injured and consequently more permeable epithelium. Oliver and Shevky's experiments with the same animal show the tubular damage to be a complete and structural one and that, in the absence of complicating vascular spasm, there is a failure of absorption rather than an increased absorption. Moore and Hellman,²³ using the intravital method of Hayman and Starr²⁴ for determining the number of open glomeruli, have shown that acute mercurial nephrosis in the rabbit is not associated with a decrease of glomerular circulation. They agree with Richards that the anuria is due to an inability of damaged tubular epithelium to prevent a resorption of tubular urine. Bieter²⁵ has demonstrated experimentally the essential rôle of glomerular injury in albuminuria. Proteinuria could be produced readily in glomerular fish (*Ameriurus nebulosus* and *Anguilla rostrata*) by asphyxia or mercuric chloride. Aglomerular fish (*Opsanus tau*) treated in the same way showed a lack of albumin, indicating that the renal glomerulus is essential for albuminuria. Very recently Jensen and Apfelbach²⁶ have reported the production of pure renal insufficiency in dogs by injection of charcoal particles into the renal arteries with resultant glomerular infarction, nitrogen retention in the blood, decrease in the ability of the kidney to concentrate and dilute urine and a lowering of its specific gravity.

We believe the histological changes in the glomerular basement membrane brought out by the special stain employed in our experiments may supply the missing link in the chain of evidence pointing to primary glomerular injury as the cause of the disturbances in renal function and blood chemistry of chemical nephropathies. Cellular damage occurs in acutely affected kidneys but is later repaired, while the splitting and fragmentation of the basement membrane is present during all stages of the disease.

The renal lesions of uranium and other metallic salts are commonly termed nephritis, a designation not properly applicable to such forms of kidney damage. The term nephritis should be restricted to those forms of renal disease exhibiting evidence of either proliferative or exudative inflammation. It is quite true that in certain chronic chemical nephropathies the interstitial connective tissue is

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DESCRIPTION OF PLATES

PLATE 109

FIG. 1. Camera lucida drawing of normal rabbit glomerulus stained with azo-carminc (oil immersion). Gl. Ep. = glomerular epithelium; End. = endothelium; Gl. B.M. = glomerular basement membrane; Rbc. = red blood cells; C.Ep. = capsular epithelium; C.B.M. = capsular basement membrane. The glomerular basement membrane is of uniform thickness, evenly stained and shows comparatively little undulation. Epithelial cytoplasm is abundant.

FIG. 2. Several loops of glomerulus from R-BQI — given 0.0875 gm. uranium nitrate intravenously over period of 148 days. Camera lucida drawing, oil immersion. 1. = lymphocytes; 2 and 3 = so-called "hyaline droplets" in cytoplasm and nuclei of capillary epithelium; Gl. B.M. = slightly split glomerular basement membrane; End. = endothelium; Rbc. = red blood cells.

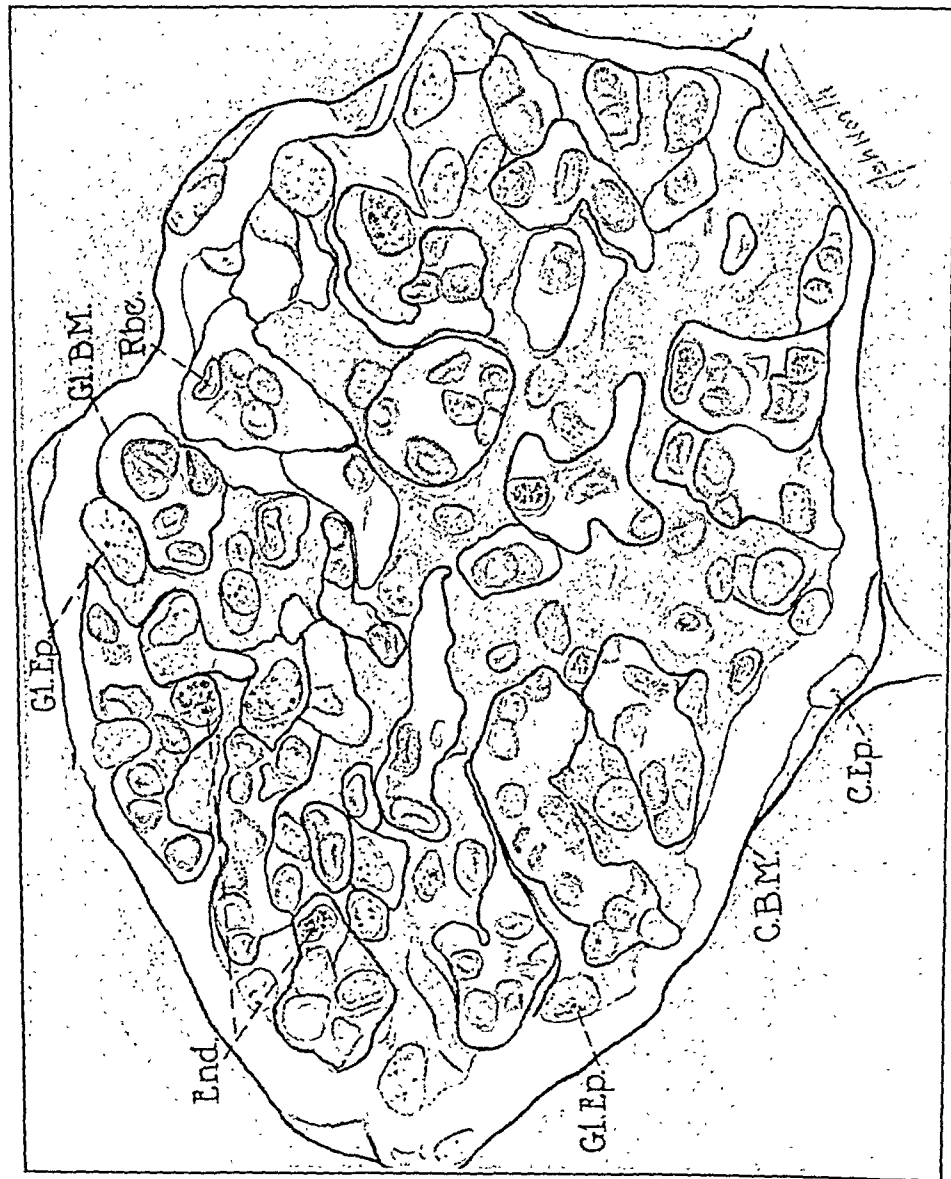
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PLATE 110

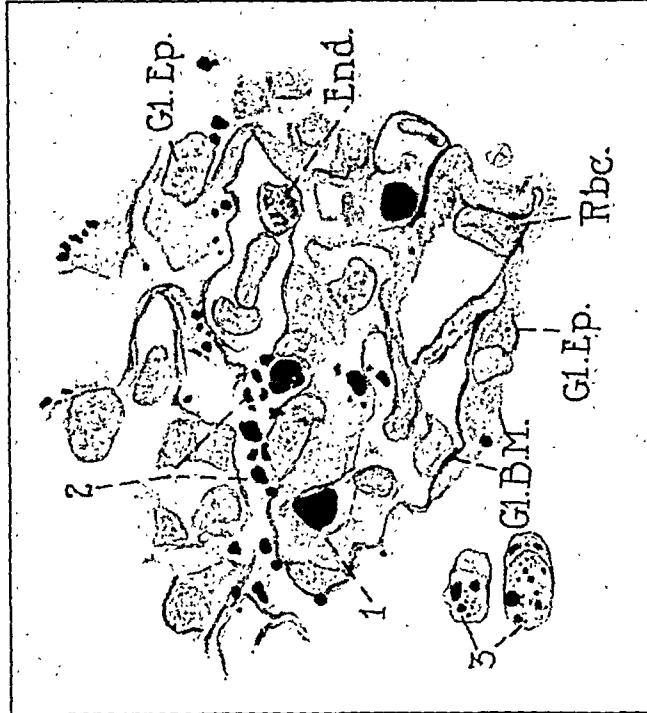
FIG. 3. Drawing of entire glomerulus of a rabbit receiving intravenous injection of 0.0005 gm. uranium nitrate and dying four days later. A small intraglomerular hemorrhage involving one loop is shown at the top with rupture of the frayed and fragmented basement membrane (Gl. B.M.) near point 2. Rbc. = erythrocytes; End. = capillary endothelium; Gl. Ep. = glomerular epithelium; 1 = lymphocytes in capillaries. (Oil immersion.)

FIG. 4. One loop of a glomerulus from rabbit with chronic uranium nephropathy displaying fraying and irregular thickening of capillary basement membrane (Gl. B.M.), and one epithelial cell (Gl. Ep.).



Hunter and Roberts

1



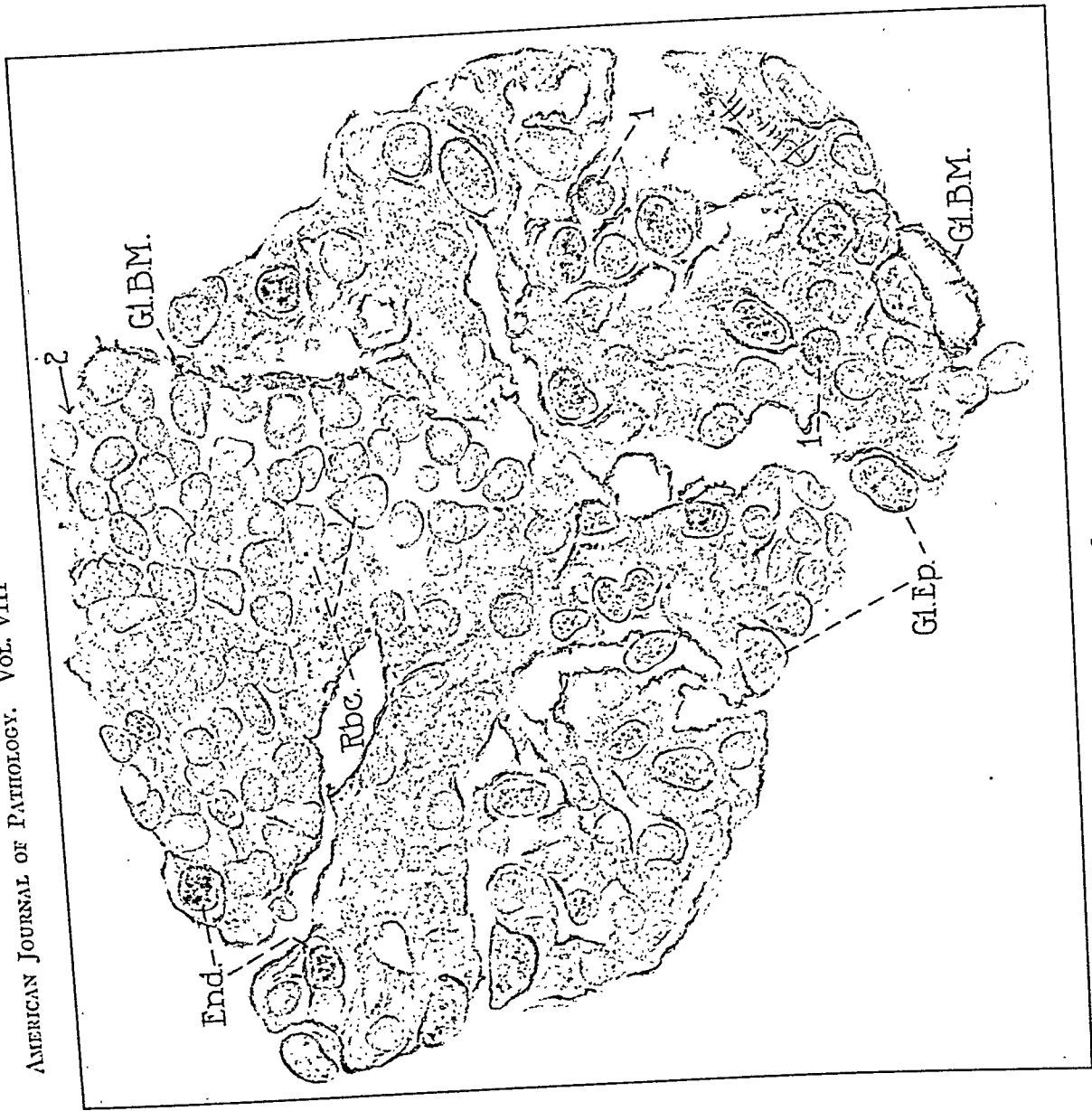
2

Glomerular Changes in Kidneys

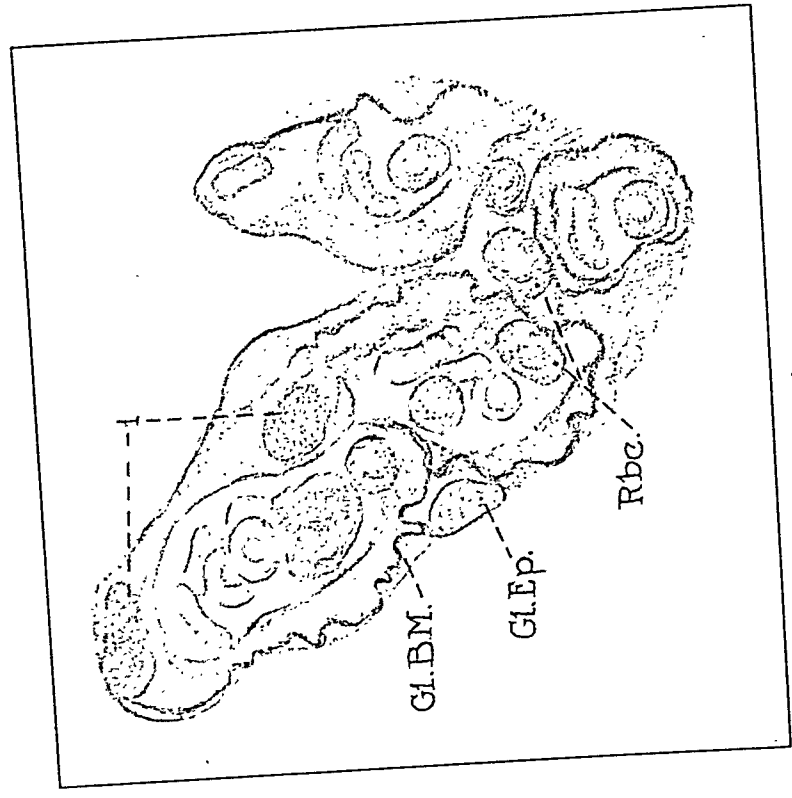
PLATE III

FIG. 5. Portions of three loops of glomerulus from R-51A (see Table II). The glomerular basement membrane (Gl. B.M.) is markedly wrinkled, and both internal and external to it is a homogeneous non-granular substance taking the same stain as the original membrane but less deeply. \mathbf{r} = nuclear inclusions within the thickened capillary wall; Gl. Ep. = glomerular epithelium; Rbc. = numerous erythrocytes in thick-walled but patent capillaries.

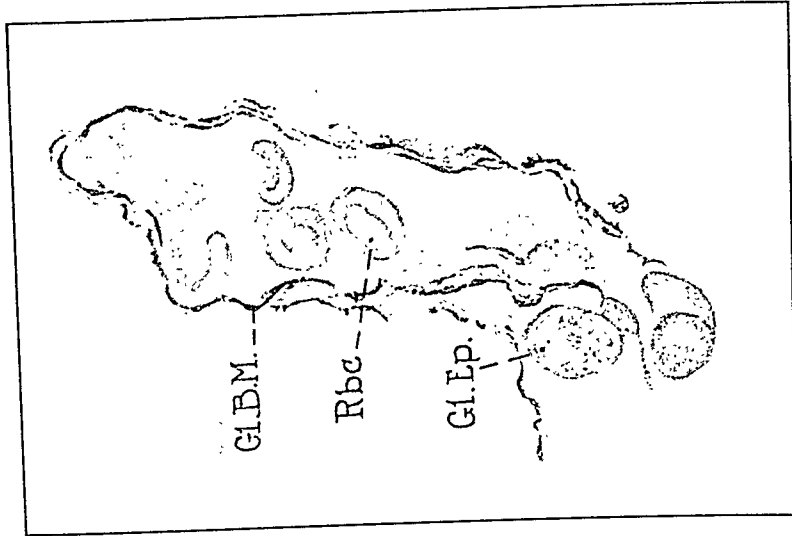
FIG. 6. Appearance of glomerulus in rabbit dying three days after subcutaneous injection of 0.026 gm. HgCl_2 . Oil immersion, camera lucida drawing. Gl. B.M. = extreme fraying and fragmentation of glomerular basement membrane. Gl. Ep. and End. = glomerular epithelial and endothelial cells both greatly reduced in number. \mathbf{r} = necrotic cellular debris filling capsular space, apparently derived from glomerular epithelium; the capillaries are distended with erythrocytes, Rbc.; C. Ep. = capsular epithelium; C.B.M. = capsular basement membrane.



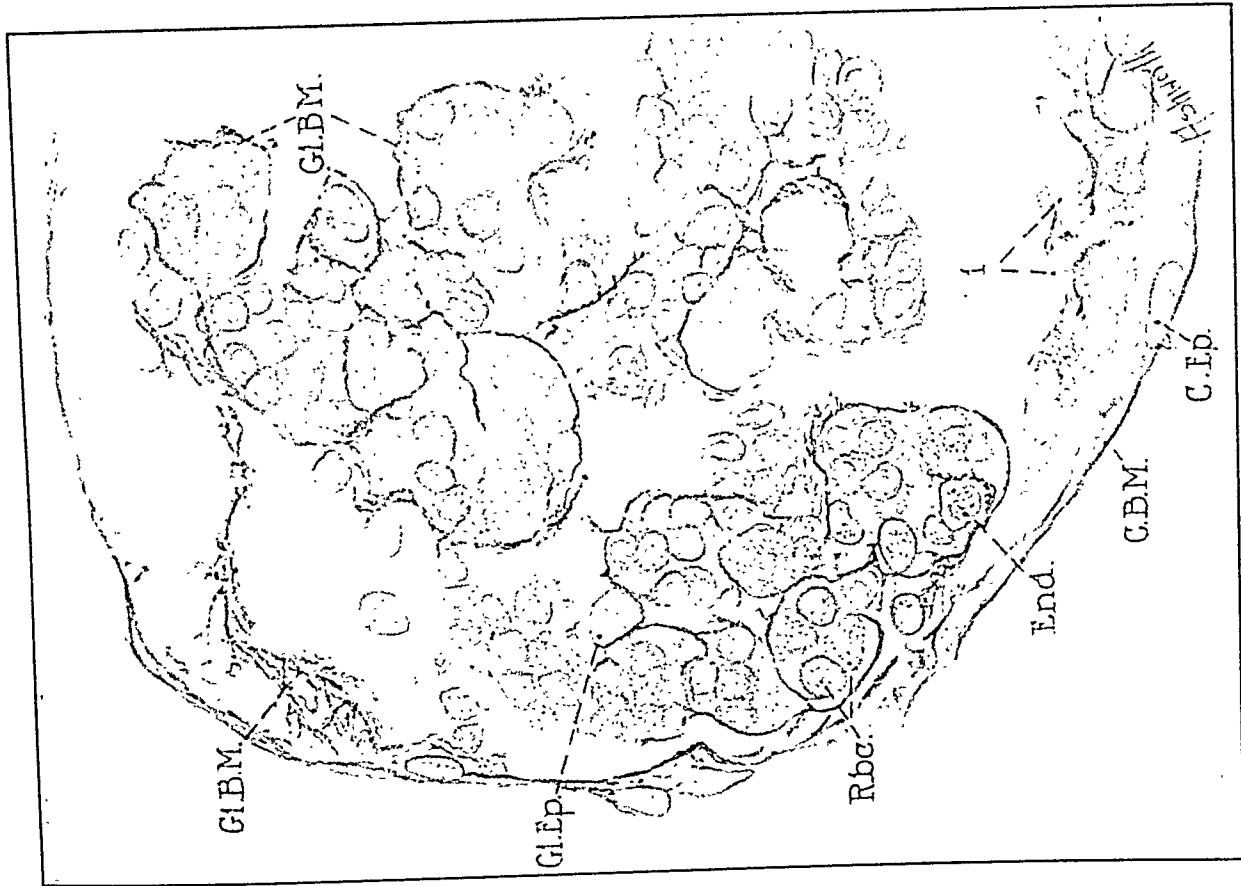
3



4



5



6

it became apparent that the bacterial nature of the antigen was not an essential determining factor in the development of the tuberculin type of allergy. It was possible to produce in tuberculous guinea pigs, with ordinary proteins such as egg-white or horse serum, a state of hypersensitivity characterized by delayed and prolonged skin reactions, an absence of demonstrable antibodies in the blood stream, failure of passive transfer, and insusceptibility to acute shock on intravenous injection. The best method to produce this type of sensitiveness was to inject the given protein directly into a tuberculous focus (a previously infected lymph node or testicle proved most convenient), and to test the animal within a period of three to ten days. The conclusions were drawn that in an infected animal a tuberculin type of hypersensitiveness to ordinary proteins could be produced, and that the distinctive characteristics of tuberculin sensitivity do not depend upon some special character of bacterial antigens or upon other secondary factors, but are the result of a special type of sensitization.

With such sharp immunological differences between the two types of hypersensitiveness it seemed only reasonable to expect that microscopic examination of the corresponding skin reactions should show clear-cut, recognizable differences. A survey of the literature, however, showed that no distinctive characteristics were generally recognized.

Descriptions of the histological features of anaphylactic reactions are found in the papers of Arthus and Breton,⁸ Gerlach,⁹ and Opie,¹⁰ most of which, however, are based upon reactions produced in rabbits. They agree essentially upon inflammatory edema, polymorphonuclear infiltration and necrosis as the characteristic findings. Gerlach also studied reactions in guinea pigs, rats, dogs and the human skin. In these he obtained somewhat slighter reactions (though his antigen doses were still very large), in which serous and polymorphonuclear infiltration predominated and necrosis was less obvious or absent, but the results were essentially similar to those found in rabbits. He described one experiment with two guinea pigs tested 4 and 6 days after sensitization which showed at 48 hours a predominantly mononuclear infiltration. This corresponds closely to findings of our own which will be described in the second part of this paper, though Gerlach made no comparison between it and tuberculin reactions.

HISTOLOGICAL STUDIES OF HYPERSENSITIVE REACTIONS *

LOUIS DIENES, M.D., AND TRACY B. MALLORY, M.D.

(From the Department of Pathology and Bacteriology of the Massachusetts General Hospital, Boston, Mass.)

PART I

THE CONTRAST BETWEEN THE HISTOLOGICAL RESPONSES IN THE TUBERCULIN (ALLERGIC) TYPE AND THE ANAPHYLACTIC TYPE OF SKIN REACTIONS

Numerous irreconcilable differences between the experimental findings of Smith, Rosenau and Anderson, Otto and the innumerable other experimenters working with protein anaphylaxis, and those of the investigators of bacterial hypersensitiveness such as Römer, Baldwin, and Krause with tuberculin, Gay and Claypole with typhoidin, Fleischer, Myer and Shaw with abortin and Helmann and Kalning with mallein pointed to the existence of two distinct types of hypersensitiveness. Zinsser¹ was the first to emphasize and clearly to express the essential points of dissimilarity which may be presented in tabular form as follows:

	ANAPHYLACTIC TYPE	TUBERCULIN TYPE
Skin tests	{ Immediate Transitory	Delayed Prolonged
Serum	{ Antibodies demonstrable Passive transfer possible	Antibodies not demonstrable Passive transfer not possible
Results of intravenous injection	{ Acute shock	Delayed shock
Sensitizing antigens	{ Ordinary proteins Bacteria and some of their protein-containing products	Bacteria, best living but also killed if in condition to produce granulomatous tissue response
Testing antigens	{ Proteins Carbohydrate fraction of bacteria (apparently the most effective)	Bacterial proteins and protein-fractions only

One of us (Dienes)²⁻⁷ in a series of studies has been able to support Zinsser's demonstration of an essential qualitative difference between the two types of hypersensitiveness. In this work, however,

* Received for publication May 15, 1932.

reactions obtained within a few days of sensitization became more and more apparent and they were eventually intensively studied, the results appearing in the second part of this paper. The study of the slight reaction, moreover, not only offers a technical advantage but also gives us more information concerning the probable rôle of hypersensitization in the economy of the organism. Under natural conditions hypersensitive tissues come in contact only with small doses of an antigen, or if exposed to larger doses, as in certain infectious lesions, they are probably desensitized.

The following types of skin reactions have been studied histologically:

1. Tuberculin reactions

- (a) True tuberculin reactions.

- (b) Tuberculin type reactions produced with egg-white or horse serum.

2. Anaphylactic reactions

- (a) Passive.

- (b) Active.

The experiments have been carried out in both normal and tuberculous animals. Guinea pigs, for the most part, were used though in some experiments a small confirmatory series was run in rabbits. In the studies upon tuberculous animals the possibility of a non-specific effect upon the histological picture was borne in mind. Kaufmann,¹⁵ for instance, found that the blisters produced by cantharidin in tuberculous individuals showed a higher proportion of mononuclear phagocytes in the exudate than similar blisters produced in normal individuals, and Sabin *et al.*,¹⁶ Medlar,¹⁷ and other investigators have noted the less directly pertinent increased mononuclear count in the peripheral blood in certain stages of tuberculosis. Indeed Selter and Tancre, ¹⁸ who noted the increased inflammatory response of tuberculous animals to many stimuli, regarded the tuberculin test itself as merely an intense form of this tendency of tuberculous animals.

Technique: The animals used throughout the experiments have been so far as possible light colored, fully grown males. Areas have been prepared for injection by careful shearing or plucking. No depilatories have been used. Several skin tests of varying age were often made upon the same pig without any noticeable desensitization. The animals were then killed, the appropriate areas cut out,

The older microscopic studies of the tuberculin reactions are somewhat contradictory. Most observers described it as a strong exudative reaction essentially like the Arthus phenomenon and minimized the difference of the anaphylactic and tuberculin types of hypersensitiveness (Spehl,¹¹ Auché and Augistrou¹²). In the German literature there are many claims that the tuberculin reaction reproduces the specific features of the tuberculous lesion (lit. see Blumenberg¹³). These claims are based upon the study of the late stages of skin reactions, several days to weeks old. The first stage of the reaction was regarded as an acute inflammatory response and received little attention. More recently Zieler and Hamel,¹⁴ the main proponents of the histological specificity of the tuberculin reaction, in a survey of the conflicting claims admitted that the microscopic structure of the reaction showed no sufficiently distinctive characteristic to differentiate it from the late stages of non-specific inflammatory processes (such as those following injection of *B. coli* vaccine), and that the only specificity of the tuberculin reaction lies in the fact that tuberculin produces its effect only in tuberculous animals.

The closely allied problem of reinfectious lesions will be discussed elsewhere.

The failure of previous investigators to find significant differences between the two types of hypersensitivity might well be explained: (1) by their choice of experimental animals, since rabbits rather than guinea pigs were usually used; (2) because strong reactions only were studied in which necrosis and the reaction to it dominated the picture, and (3) because the tuberculin reaction was not studied in the first few hours of its development when its special characteristics are most apparent.

We decided to reattack the problem, making use of the methods of sensitization developed by Dienes in his former studies. We have devoted our attention particularly to reactions of moderate and even slight intensity, finding in the course of our study that in these weaker reactions the differences between the two types were most readily observed. Two procedures were available for producing such reactions, both of which we have made use of: (1) the testing of highly sensitive animals with minimal test doses, and (2) the testing of very slightly sensitive animals with larger doses.

In the pursuit of this second method the significance of the early

reddish rings 2 to 3 cm. in diameter. At 48 hours the central zone is yellow-brown in color, the surrounding ring entirely red. From 72 hours on the brownish area dries up, forming a scab which eventually sloughs and falls off. The interval before complete healing is very variable.

In very slight reactions only a red area of induration with some central blanching may be made out grossly. By increasing the test dose in animals whose degree of sensitization is slight, extensive reactions up to 4 cm. in diameter may be obtained which show no necrosis. In contrast, well marked necrosis may be obtained in highly sensitive pigs with a dose so small that a lesion only 1 cm. in diameter is produced. The development of necrosis is therefore independent of the size or extent of the lesion and must depend upon some difference in the reaction mechanism.

An experience with the microscopic appearances of several hundred tuberculin reactions may be described in the following composite picture. As early as 2 hours after injection of the tuberculin, before any trace of reaction is visible grossly, the fixed tissue cells of the corium, both endothelial and fibroblastic, have become more prominent than normal and there is a well marked infiltration of large mononuclear phagocytes, with practically no admixture of polymorphonuclears.

At 6 hours the mononuclear infiltration is much more marked, appearing in part diffusely but being most evident in focal collections situated in the adventitia of small vessels and nerves. Polymorphonuclears may be present in moderate numbers, reaching in extreme instances 40 per cent of the invading cells, but more commonly comprising not over 10 to 15 per cent of the total. Evidence of necrosis cannot usually be made out up to this time. The absence of edema is quite striking in comparison with anaphylactic tests.

From 7 to 12 hours, in the stronger reactions, an alteration in the staining of the cells of the basal layer of the epidermis becomes apparent. The nuclei may become pyknotic. The cytoplasm is less strongly basophilic and often becomes vacuolated. A zone of polymorphonuclear infiltration appears just beneath this degenerating layer.

By 24 hours the necrosis of the epithelium is usually obvious and extensive. In the less severe reactions evidence of necrosis is sharply limited to this layer and a narrow zone of underlying derma. In the

pinned upon corks, fixed in formalin and embedded in paraffin. Several sections were prepared from each block beginning at the center of the reaction and working outward at 2 to 3 mm. intervals. The sections were stained with hematoxylin and eosin.

Controls: In normal animals an intracutaneous injection of normal saline alone will produce a definite reaction. In the track of the needle there is a slight, rapidly developing necrosis and transitory polymorphonuclear infiltration. It is almost wholly limited to the needle track, however, and the surrounding tissue — the zone particularly studied in our experiments — shows only the faintest trace of polymorphonuclear and mononuclear infiltration. Egg-white, horse serum and synthetic tuberculin in the doses used show no more.

In many tuberculous animals the slight injury caused by the injection of normal salt solution or of egg-white or horse serum caused a much more definite reaction with edema and some initial polymorphonuclear infiltration. By 24 hours there was a slight but well marked predominance of mononuclears and the reaction was sometimes indistinguishable from a minimal tuberculin response. The variations between individual animals were sufficient to make it desirable to examine a control of NaCl or indifferent serum in each animal tested. Under these conditions no difficulty was experienced in judging the non-specific element of the reaction. As a further control of the effect of the tuberculous infection turpentine was injected intracutaneously into both normal and tuberculous animals. In the former an intense polymorphonuclear infiltration occurred. In the latter this was quite as intense but a slight increment of mononuclear infiltration was noted. The tuberculous infection evidently in no way inhibited the leukocytic exudation.

THE HISTOLOGY OF THE TUBERCULIN REACTION

The gross and microscopic appearances of tuberculin reactions vary enormously with the degree of sensitivity of the animal, its general condition, and the size of the test dose. A typical, moderately strong reaction might be expected to show the following gross changes. The bleb caused by the injection disappears in a short time. At 3 to 6 hours there is a slight, firm swelling with accompanying redness. At 12 hours a central necrotic spot begins to be apparent. By 18 to 20 hours a circumscribed violet (hemorrhagic) center 0.5 to 1 cm. in diameter is seen surrounded by indurated white and

Tuberculin Type Lesions Produced with Egg-White

Tuberculous animals sensitized according to the method of Dienes by injecting a protein such as egg-white or horse serum directly into a tuberculous focus showed on skin test 3 to 10 days later a type of skin reaction corresponding in every detail of gross and microscopic appearance with the usual tuberculin tests described above. There was the same mononuclear predominance up to 6 hours, and again after 48 hours, with an intermediate period of relative polymorphonuclear increase at approximately the same period as the development of epithelial necrosis.

Since strong reactions of this type could be produced only in tuberculous animals it was obviously necessary to bear in mind the non-specific tendency of tuberculous animals to react to all inflammatory stimuli with a heightened mononuclear response. The degree of mononuclear infiltration in our test animals was, however, far greater than that observed in the non-specific control reactions. Moreover the further experiments recorded below and also those in Part II serve as added controls. In these we will show that an anaphylactic response produced in a tuberculous animal is characterized by only a slight mononuclear increase over the reaction produced in normal animals, and also that in non-infected animals a typical tuberculin type of reaction, albeit a weak one only, can be produced which shows particularly clearly the mononuclear predominance.

ANAPHYLACTIC TYPE SKIN REACTIONS

For reasons which will become evident in the second part of this paper skin tests upon passively sensitized animals present a sharper histological contrast to the tuberculin type of reaction than do tests upon actively sensitized animals. To emphasize this contrast the passively sensitized animals will be discussed first.

Anaphylactic Type, Passive Sensitization

Technique: The animals, guinea pigs and rabbits, were passively sensitized with strong homologous sera, anti-egg-globulin in the case of the pigs, anti-egg-white in the case of the rabbits, and skin tested with 0.1 mg. a few days later. Egg-globulin was selected for some

more severe reactions a hemorrhagic type of necrosis of the underlying connective tissue may be fairly extensive. At this time the mononuclear predominance in the formula of the invading leukocytes is much less marked, and the polymorphonuclears may rise to 60 to 70 per cent of the invading cells. The mononuclears persist in large numbers, however, and even at this stage are far more numerous than at any period in the anaphylactic type of reaction.

By 48 hours regenerating epithelial cells can be seen growing in from the edges of the lesion beneath the necrotic remnants of the original epithelium, but above the underlying zone of connective tissue infiltrated with leukocytes. The polymorphonuclears are now sharply restricted to this zone and the deeper and peripheral parts of the lesion show an almost purely mononuclear infiltration. A considerable proportion of the polymorphonuclears are beginning to show evidences of degeneration, including fragmentation. The mononuclears on the other hand appear relatively uninjured and many of them contain phagocytosed débris of the necrotic polymorphonuclears. In many the cytoplasm is somewhat swollen, more acidophilic than in the earlier stages, and more suggestive of the "epithelioid" appearance of the cells in true tubercular lesions.

In summary then, in the average tuberculin reaction of moderate intensity, a wandering cell infiltration predominantly mononuclear in character occurs. The mononuclear predominance is greatest in the early stage, up to 6 hours, and again in the late stages from 48 hours on. During the intermediate stage from about 7 to 48 hours the proportion of polymorphonuclears increases but rarely becomes predominant. During approximately this same period signs of degeneration and necrosis of the epithelium occur and the zone of polymorphonuclear infiltration is most intense in the neighborhood of the degenerating epithelial cells.

In less severe reactions, either in highly sensitive animals tested with a very small dose, or in recently inoculated animals in which the sensitiveness is not fully developed, the mononuclear predominance is still more marked at all stages up to 48 hours. In these reactions evidence of necrosis can rarely be made out.

The findings suggest, but are not sufficient absolutely to prove, that the polymorphonuclear infiltration evident in the middle period of the moderately strong reactions is in part, at least, secondary to necrosis of epithelium.

above 15 per cent) was noticeable at 1 and 6 hours, but at 24 and 48 hours a definite mononuclear infiltration was evident. This, however, was no more marked than that of the salt solution controls.

Anaphylactic Type, Active Sensitization

Technique: The two animals used in these experiments were tuberculous. Egg-white was injected first into the tuberculous lesions and 6 days later 15 mg. were given intravenously. On subsequent skin tests they showed strong reactions of the anaphylactic type, whereas another group similarly treated, except for the intravenous injection, showed the tuberculin type of reaction. Two months later, after repeated skin tests, they were retested with 0.01 mg. ($\frac{1}{10}$ the usual dose) and the reactions were examined at 1, 6, 24 and 48 hours.

Grossly both pigs showed at 1 hour a wheal 16 mm. in diameter which increased to 22 mm. at 6 hours, then rapidly faded to a trace of redness without swelling at 24 hours.

Microscopically at 1 hour there was marked edema and a strong infiltration with polymorphonuclears, both diffuse and in clusters in the corium. The proportion of mononuclears was very small.

At 6 hours the edema and polymorphonuclear infiltration were still more marked. The proportion of mononuclears was slightly greater but still small in proportion to the number of granulocytes. No morphological evidence of necrosis was observed.

At 24 hours the edema and polymorphonuclear infiltration had largely disappeared, except for a small central zone in the traumatized area. A diffuse, fairly marked, predominantly mononuclear infiltration of the loose subcutaneous tissue was present, which did not, however, show the closely packed accumulations of mononuclears found in the tuberculin type reactions.

In other experiments reactions macroscopically and microscopically similar were observed in non-infected, actively sensitized pigs.

SUMMARY AND DISCUSSION

The gross and histological pictures of the skin reactions in the anaphylactic and tuberculin types of hypersensitivity have been described and contrasted. The differences were found to be particularly emphasized in reactions of slight intensity on the one hand, and

of these tests because various proteins show relatively different powers of producing one or the other type of sensitivity. Egg-albumin, for instance, more readily produces the anaphylactic type, egg-globulin the tuberculin type. Therefore an anaphylactic type of reaction produced with egg-globulin is especially significant in demonstrating that the method of sensitization is more important than the chemical nature of the antigen. The egg-globulin preparation was of sufficient purity so that no precipitate was obtained with egg-albumin serum and that it failed to produce shock in animals sensitized with egg-albumin. The ovomucin, however, was not removed.

In such passively sensitized animals skin tests produced a rapidly developing wheal reaching in 1 hour 16 to 20 mm. in diameter with a peripheral pink or red zone of variable width. The reaction persisted up to 6 hours with but slight diminution in intensity, then faded quickly away. At 24 hours no trace remained except for the pin-point scar of the needle prick.

The microscopic appearances were quite different from those of the tuberculin tests. At 1 hour the blood vessels were dilated, the corium showed a marked diffuse edema, and polymorphonuclears were scattered in large numbers throughout the tissue. No increase in mononuclear elements could be made out.

At 6 hours the congestion and edema had slightly decreased. The polymorphonuclear infiltration on the other hand was more dense and a small number of large mononuclears had appeared. At 24 hours almost no trace of reaction was left except for a few scattered mononuclears.

In normal rabbits passively sensitized with anti-egg-white serum the gross reaction with 0.1 mg. of egg-white was less obvious and somewhat slower in development. By 6 hours, however, a small, soft, swollen, reddish spot was evident, which had decreased at 24 hours to a trace of redness. Microscopically the 6 hour reaction showed marked edema and dense infiltration with characteristic pseudo-eosinophilic polymorphonuclears and small numbers (5 to 10 per cent only) of mononuclears.

In the tuberculous animals similarly treated no gross difference in the reactions could be made out except that the local scar of the needle puncture was a trifle more noticeable and more persistent. Microscopically a slight increase of mononuclear leukocytes (not

derlying factor in the stage of anaphylactic hypersensitiveness, so that what we are observing is really a mixed reaction with a predominance of the anaphylactic type.

Necrosis, though it occurs in the stronger anaphylactic type of reactions as described by Arthus and Opie, was not obvious in our experiments since the doses were purposely kept low. When it occurs it is, according to the observations of Opie and Gerlach, most marked in the fibroblastic and endothelial elements rather than in the epithelium. In tuberculin sensitivity, on the other hand, necrosis is the rule if the animal be highly sensitized even when the test dose is very small. Though in severe reactions extensive necrosis of all tissue elements may occur, in the slighter reactions it seems to be more or less selectively limited to the epithelium.

In this connection the results obtained by Aronson¹⁹ in tissue cultures are of interest. He found that cultures prepared from tuberculous animals were killed by the addition of tuberculin, whereas cultures prepared from anaphylactically sensitive animals were uninjured by the addition of the appropriate antigen.

In the tuberculin type of hypersensitiveness the contact of cells and antigen starts an entirely different biological process from that produced in the anaphylactic type.

CONCLUSIONS

A histological comparison of the skin lesions of the anaphylactic and the tuberculin types of hypersensitiveness under appropriate conditions permits us to add to the already recognized differential points: (1) an initial hyperemic and exudative phase in the anaphylactic type contrasted with an initial phase of cellular infiltration in the tuberculin type; (2) a tendency to a predominantly polymorphonuclear leukocyte infiltration in the anaphylactic, compared with a predominantly large mononuclear infiltration in the tuberculin type, and (3) a tendency to selective epithelial necrosis in the tuberculin type, not observed in the anaphylactic type.

in animals with relatively low sensitivity on the other. At all stages, however, recognizable differences between the two types occur, and these differences must be considered qualitative not quantitative.

The tuberculin type of reaction, whether produced with tuberculin or, in an appropriately treated animal, with a protein such as egg-white or horse serum, is characterized by the slow development of exudative phenomena and the early infiltration of mononuclear phagocytes. At a later period, from 12 to 24 hours, necrosis, most marked in and sometimes selectively localized to the epithelium appears. At the same period a marked increase in polymorphonuclear infiltration occurs, the temporal concurrence and the localization to the neighborhood of the degenerating epithelium suggesting that the leukocytic invasion may be primarily a reaction to necrosis. In tuberculin tests upon animals of relatively low sensitivity necrosis may not develop even in extensive reactions produced with large doses. In such reactions polymorphonuclear infiltration also remains slight.

In passively produced anaphylactic type reactions is found the sharpest contrast to this picture. In such reactions a rapidly developing edema, quickly followed by a fairly intense polymorphonuclear infiltration are the first phenomena observed. This reaction reaches its height somewhere between 1 and 6 hours and then rapidly fades away. At 24 hours a few persisting mononuclears are the only trace of the reaction.

In passive anaphylactic reactions produced in tuberculous animals a slight increase in the proportion of mononuclear phagocytes is noted. This is, however, no greater than the mononuclear reaction produced by indifferent sera or by salt solution in the same animals and does not significantly affect the marked polymorphonuclear predominance in the formula of the invading wandering cells.

In actively produced anaphylactic reactions, whether in normal or tuberculous animals, there is again a slight infiltration with mononuclears, evident at 6 hours and predominant at 24 hours. Their number, however, remains far below that seen in tuberculin type reactions and they fail to show the perivascular and perineural clusters so characteristic of that type.

Part II of this paper will present evidence for believing that in actively sensitized animals a certain amount of the tuberculin type of hypersensitiveness may develop along with, or persist as an un-

groin in the usual fashion. The uninfected guinea pigs were sensitized intraperitoneally. Skin tests in all instances were done at short intervals, from 3 to 6 days after sensitization.

EXPERIMENT I

Tuberculous guinea pigs sensitized with egg-white, tested on the third and fourth days.

TABLE I

2 Hour and 24 Hour Readings of Skin Tests Performed on Tuberculous Guinea Pigs on 3rd and 4th Days after Sensitization

Guinea pig No.	March 13	March 16		March 17	
	Sensitization	Skin tests with 0.2 mg. egg-white i.c.		Skin tests with 0.2 mg. egg-white i.c.	
		2 hr. reading	24 hr. reading	2 hr. reading	24 hr. reading
308	3 mg. egg-white i.p.	Tr. white	20 × 20 red tr. sw.		
311	"	Neg.	12 × 12 tr.		
309	"			Tr. red and sw.	Sl. sw. ¹
314	"			Tr. red and sw.	12 × 21 bright red
307	Unsensitized control			Tr. red	Tr. red
310	"			Tr. red	10 × 10 tr. ²

In this and the following tables the numbers signify mm., sw. = swelling, sl. = slight, tr. = trace.

All animals had received on March 5 (8 days before sensitization) 20 mg. R1 tubercle bacilli intraperitoneally.

¹ Guinea pig had a dark skin obscuring redness.

² Site of injection scratched.

Histology of Skin Lesions: The reactions observed were typical of rather mild tuberculin reactions, with a strong mononuclear infiltration, very few polymorphonuclears, no obvious necrosis. The appearances do not differ qualitatively from those observed in uninfected animals but are quantitatively distinctly more intense. The unsensitized tuberculous animals used as controls showed very slight infiltration which was predominantly polymorphonuclear in character.

PART II

THE TUBERCULIN (ALLERGIC) TYPE OF RESPONSE IN THE FIRST PERIOD OF SENSITIZATION AND ITS RELATION TO THE PROCESS OF IMMUNIZATION

In the introduction to the first part of this paper we emphasized the importance of studying relatively mild reactions in order to obtain the sharpest differentiation between the anaphylactic and the tuberculin types of reaction. One of the methods used was the study of early skin tests, shortly after sensitization, when hypersensitivity was as yet incompletely developed. The study of these early reactions, moreover, served not only to emphasize the differences in histological reaction in the two types of hypersensitivity, but to throw an entirely new light upon the significance of the tuberculin type of hypersensitiveness by showing that it is a usual and perhaps universal stage in the immunological response of the organism to foreign protein injection.

When animals, tuberculous or uninfected, are tested with ordinary protein antigens within a short period (3 to 6 days) of sensitization, specific skin tests are obtained which show all the essential criteria of the tuberculin type of hypersensitivity. This has been pointed out before by Dienes in the case of appropriately treated tuberculous animals in which the gross appearances of the reactions are characteristic enough to determine the nature of the hypersensitivity. In the case of uninfected animals such strong reactions are never obtained. Grossly there is only the delayed development of slight hyperemia and induration which persists for 48 to 72 hours. On microscopic examination these reactions show the same mononuclear predominance described in our first paper as characteristic of the tuberculin type of sensitivity, and with this additional criterion we feel that there can be no doubt as to their classification.

Our experiments fall into two groups, the first performed upon tuberculous animals, the second upon uninfected ones. The tuberculous pigs were prepared by intraperitoneal infection and subsequent injection of the protein antigen into the peritoneal cavity — a method equivalent to direct injection into a tuberculous lesion. The rabbits were inoculated directly into a tuberculous node in the

predominate. In the control reactions the infiltration is very much less extensive and polymorphonuclears make up 60 to 80 per cent of the invading cells.

TABLE III

4 Hour and 24 Hour Readings of Skin Tests on Uninfected Guinea Pigs 6 Days after Sensitization with Egg-white or Horse Serum

Guinea pig No.	December 22	Skin tests December 28				
	Sensitization	Egg-white 0.2 mg.		Horse serum 0.005 cc.		Tuberculin
		4 hr. reading	24 hr. reading	4 hr. reading	24 hr. reading	24 hr. reading
68	5 mg. egg-white i.p.	Neg.	10 × 10 red	Neg.	Neg.	Neg.
69	"	Neg.	20 × 22 red	Neg.	Neg. ¹	Neg.
70	0.1 cc. horse serum i.p.	Neg.	Neg.	Neg.	12 × 10 red	Neg.
71	"	Neg.	Neg.	Neg.	14 × 12 red	Neg. ¹

¹ These animals showed a trace of redness about the puncture wound.

This experiment was twice repeated with similar results, both grossly and histologically. For histological purposes these animals were killed 24 hours after skin testing. In other similarly treated animals, not sacrificed, the area of induration and redness persisted 48 hours before beginning to fade. Delayed and prolonged reactions of this type have been obtained as early as the third day after injection.

At 24 hours the infiltration is much more intense and averages 85 to 90 per cent mononuclear. No necrosis was observed in these reactions except in the line of the needle puncture.

SUMMARY OF EXPERIMENTS

Experiments have been recorded describing the skin reactions in sensitized animals, both tuberculous and uninfected, in the earliest detectable stage of hypersensitivity. It has been shown that the skin tests of this first phase of allergy show all the essential characteristics, not of the anaphylactic type, but of the tuberculin type of hypersensitivity. They are delayed in appearance, showing nothing grossly before 6 hours; they persist 48 hours or longer, and microscopically they show a predominantly mononuclear cellular infiltration in contrast to the polymorphonuclear infiltration characteristic of the anaphylactic type. The reactions observed in uninfected animals do not differ qualitatively from those of tuberculous animals but are quantitatively distinctly less intense.

EXPERIMENT II

Tuberculous rabbits sensitized with egg-white and tested 3 and 5 days later.

TABLE II

2 Hour and 24 Hour Readings of Skin Tests on Tuberculous Rabbits Sensitized with Egg-white 3 and 5 Days after Sensitization

Rabbit No.	Skin tests on 3rd day		Skin tests on 5th day	
	3 hr. reading	24 hr. reading	3 hr. reading	24 hr. reading
165	Neg.	16 × 13 tr. red		
167	Neg.	16 × 13 tr. red		
164			Neg.	28 × 23 bright red
163			Neg.	Small whitish
Controls			Neg.	Neg.

The four rabbits had been twice injected in the groins with the R₁ tubercle bacillus strain. When a large swelling appeared 2.5 mg. of egg-white were injected into the swollen tissue. The dose used for skin testing was 0.5 mg.

In other experiments, to be fully described elsewhere, tuberculous rabbits showed no skin reaction 2 days after sensitization, but a well marked reaction in 4 days.

The microscopic findings in these rabbits were essentially similar to those of the guinea pigs, a marked mononuclear infiltration being the predominant feature.

EXPERIMENT III

Uninfected guinea pigs sensitized and tested on the sixth day with egg-white or horse serum.

Microscopically these lesions show at 6 hours, the time at which they first become macroscopically visible, a marked infiltration with mononuclear cells. This is particularly marked in the immediate subepithelial layer and also in the loose tissue between the corium and the muscularis. Polymorphonuclears may be almost absent or present in moderate numbers, the usual formula being about 80 to 90 per cent mononuclears to 20 to 10 per cent polymorphonuclears. In rare instances the latter may rise almost to 50 per cent but never

sensitization with different proteins, all are characteristic of the tuberculin type of sensitivity, and we feel justified in concluding that it is a manifestation thereof. The tuberculous infection, therefore, is not essential to the development of this type of allergy, but serves merely to intensify it and also under some circumstances to prolong it and put off or prevent the appearance of the anaphylactic type.

Tuberculin hypersensitiveness, as we observe it in the course of a tuberculous infection, represents a strong and persistent development of the first stage of the sensitization process made possible by the special conditions created in the organism by the infection.

In this early stage of sensitization antibodies cannot be demonstrated in the blood stream by precipitin tests or complement fixation. If circulating antibodies are involved in the mechanism of this type of hypersensitivity they must be of an unknown nature or else exist in the blood stream in some as yet undetectable form. It appears more probable that we are dealing with an altered tissue reactivity, as specific to the protein antigen arousing it as are the usual serum reactions.

The conception of the relation between the tuberculin type of hypersensitiveness and the usual protein sensitization developed above resembles closely the often expressed opinion that tuberculin sensitivity is due to antibodies fixed upon the cells, whereas in the anaphylactic type circulating antibodies are responsible. Bessau and Detering,²¹ moreover, after showing that in children injected with horse serum marked skin sensitiveness may develop before antibodies appear in the circulation, expressed the opinion that this early stage of sensitization corresponds with tuberculin hypersensitiveness. Our conception is essentially in accord with theirs, but with animal experimentation it was possible to obtain more extensive evidence in its support.

Scattered observations entirely consistent with those we have reported are not infrequent. The finding by Gerlach⁹ of predominantly mononuclear infiltration in two guinea pigs tested on the fourth and sixth days after sensitization has already been mentioned in the introduction to our first paper. Redfern,²² moreover, described reactions in the human skin, produced by reinjecting horse serum after a relatively short interval into the original sensitizing site, which were characterized by an infiltration of round cells which he regarded as lymphocytes. Spohl,¹¹ in a description of the his-

DISCUSSION

In Part I of this paper we showed that a sharp difference was recognizable in the histological appearances of the skin reactions in the anaphylactic and the tuberculin types of hypersensitiveness. The former was characterized by a highly transitory but fairly intense serous and polymorphonuclear exudation, the latter by a slow and relatively persistent mononuclear infiltration. These differences were particularly sharp in the less intense reactions, the maximal contrast being obtained between a passively sensitized anaphylactic animal and an early tuberculin test upon an animal still in a low degree of hypersensitivity. It was noted that in actively sensitized anaphylactic animals the contrast, though still readily discernible, was not so sharp, since a slightly higher proportion of mononuclears was evident at all stages than in the passively sensitized animals and the lesion was a little less rapidly evanescent. We felt justified in concluding that the mononuclear-polymorphonuclear ratio could be added to the already known criteria of differentiation between the two types of hypersensitivity.

In tuberculous animals it had already been found possible to produce at will an anaphylactic or a tuberculin type of hypersensitivity to the same protein (such as egg-albumin, egg-globulin or horse serum) by suitable variations in experimental technique. By the third day after sensitization a slight but definite tuberculin type of sensitivity could be demonstrated which increased in intensity for several days. After a variable period of time, greatly influenced by the mode of sensitization, the stage of the tuberculous infection, subsequent protein injections even as skin tests, and other factors which will be discussed at length elsewhere,²⁰ the anaphylactic stage of hypersensitiveness may supervene. Whether or not a certain degree of persistence of the tuberculin type of hypersensitiveness occurs after the development of the anaphylactic type is uncertain, but is suggested by the higher ratio of mononuclears in the skin tests upon actively sensitized as against passively sensitized animals.

In the present paper skin tests upon uninfected sensitized animals performed in equally early stages of sensitization have been described and found qualitatively similar though quantitatively less intense. The delayed reaction, the prolonged character, the mononuclear predominance, the specific character, as proved by cross-

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tology of tuberculin reactions mentioned the early necrosis of the epithelium and also noted that at certain stages the infiltrating wandering cells were mostly mononuclear. Perhaps, also, the histological finding in reinoculation experiments with vaccinia virus of a mononuclear infiltration of the corium is evidence of a similar mechanism in the field of the virus diseases.

The significance of such a conception to immunological theory and the morphology of infectious lesions is evident. If a specific tissue response occurs as early as the third day after parenteral introduction of an antigen, a tuberculin type of allergy may play a rôle in the cellular response to a great variety of infectious agents.

It is interesting to recall in this connection that the four diseases in which this type of hypersensitivity has long been recognized — tuberculosis, glanders, typhoid and the *Brucella* infections — are all diseases in which mononuclear phagocytes, at least in certain stages, dominate the cellular response. The assumption has often been made in the past that the granulomatous nature of the infection might be the cause of the delayed type of hypersensitiveness shown in these diseases. The experiments described in this paper at least suggest a converse relationship, that a tuberculin type of hypersensitivity may be the determining factor in the mononuclear infiltration.

CONCLUSIONS

1. The tuberculin type of hypersensitiveness represents the first stage of the immune response to parenterally introduced protein antigen.
2. It occurs in uninfected as well as in tuberculous animals.
3. A tuberculous infection quantitatively increases it but does not alter it qualitatively.
4. It may be demonstrable as early as the third day after sensitization.

DESCRIPTION OF PLATES

PLATE 112

FIG. 1. A tuberculin reaction of slight intensity produced on the fourth day after infection by the intracutaneous injection of 0.01 cc. of synthetic tuberculin. The lesion was excised 6 hours after the injection.

The reaction consists of a predominantly mononuclear infiltration most marked in the perivascular tissues. There is no apparent edema or necrosis.

FIG. 2. A tuberculin type of reaction produced with 0.2 mg. of egg-white in a tuberculous rabbit 4 days after sensitization by the injection of 5 mg. of egg-white directly into tuberculous lesions.

The morphological character of the lesion is in all respects similar to that of the true tuberculin reaction illustrated in Fig. 1.

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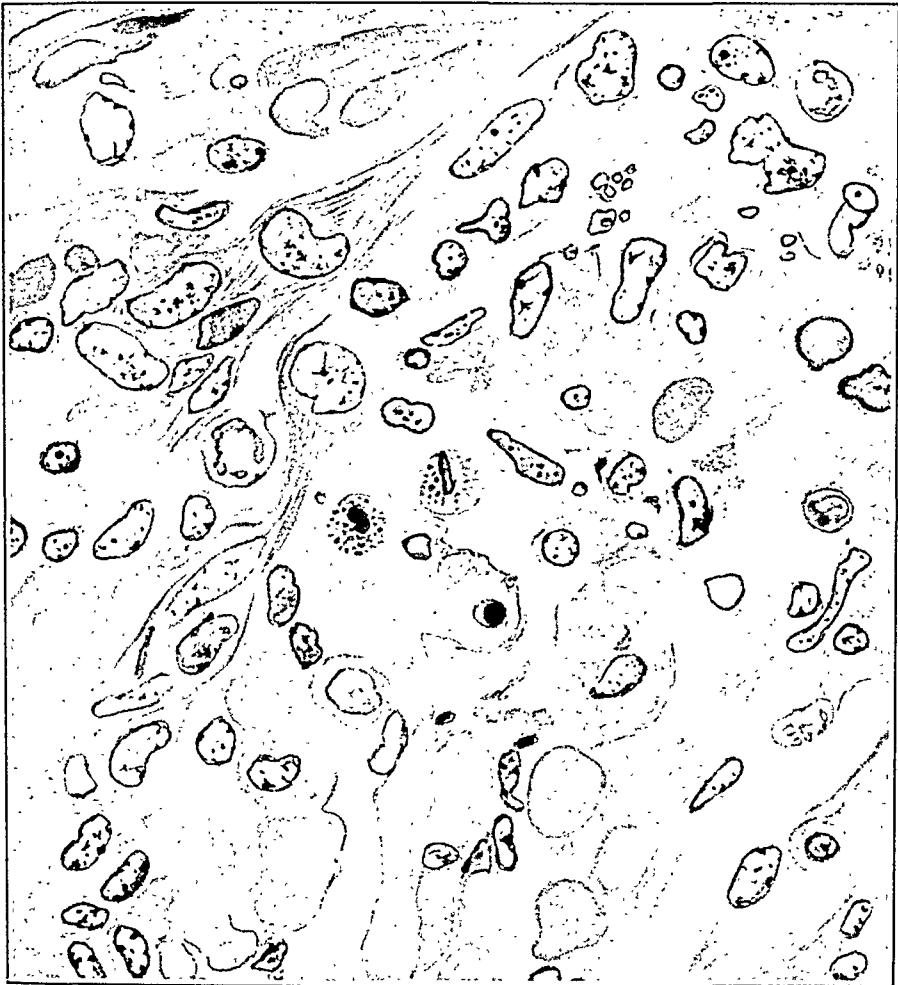
PLATE 113

FIG. 3. An anaphylactic type of reaction produced in a passively sensitized rabbit 6 hours after the intracutaneous injection of 0.2 mg. of egg-white.

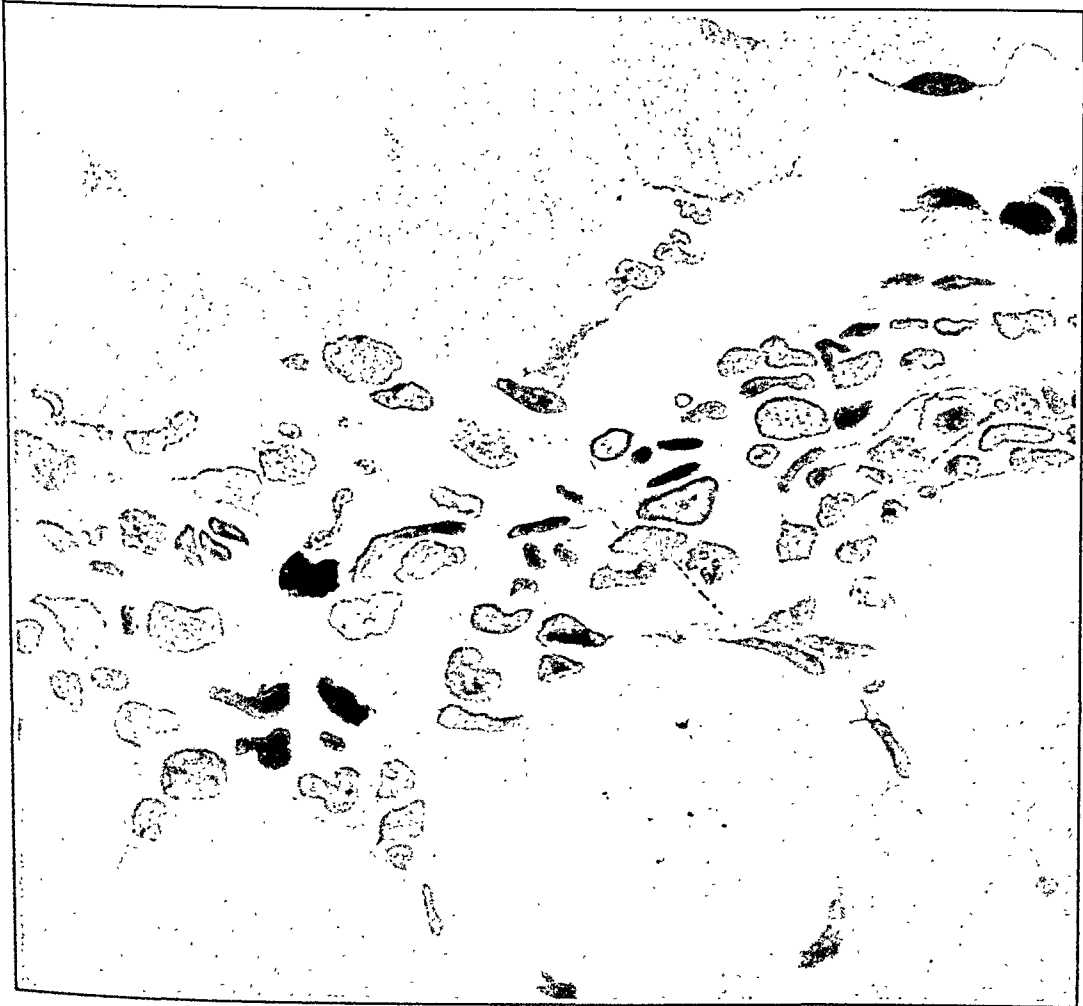
The diffuse edema and the predominantly polymorphonuclear character of the invading leukocytes is evident.

FIG. 4. A reaction produced in an uninfected guinea pig by the intracutaneous injection of 0.2 mg. of egg-white. The pig had been sensitized 72 hours before by intraperitoneal injection of 2 mg. of egg-white. The lesion was excised 6 hours after the injection.

The mononuclear response, most marked in the perivascular tissues, is characteristic of the tuberculin type of sensitization.



I



2



3



4

vesicular and disappeared. The Golgi apparatus was fragmented and there was an extrusion of nucleolar material.

Woodruff and Goodpasture,⁵ in a series of interesting experiments, isolated inclusion bodies from the epithelial lesions by tryptic digestion and were able to reproduce the disease by inoculation with a single inclusion body obtained by using Chambers' micropipette apparatus. They concluded that the inclusions were composed of minute bodies which represented the virus. Later these authors, by breaking up the inclusion body, demonstrated the infectivity of the "Borrel bodies," as compared with the non-infectivity of the lipoidal components of the inclusion.

Because of the possibility that viruses may be adsorbed onto the inclusion body, any information which can be obtained concerning the chemical nature of the inclusion should be of value. Furthermore, a knowledge of the chemical make-up of the inclusion may yield data concerning its origin from the various cell constituents. With this in mind, Scott⁶ applied the technique of microincineration to the study of the nuclear inclusions found in the submaxillary glands of guinea pigs. Later, Covell and Danks,⁷ while investigating the nature of the Negri body, made use of the same technique and found that the Negri body contains certain inorganic constituents. They suggest from these findings that the Nissl substance and the basophilic nuclear chromatin are principally concerned in the formation of the Negri body. The inclusion of fowl-pox being an extremely large one makes it favorable material for a chemical examination. It was decided, therefore, to apply this method to the inclusions of fowl-pox and ascertain what inorganic constituents, if any, they contain. The microincineration technique used was that devised by Policard⁸ and more recently modified and applied to the study of cytological problems by Scott.⁹

Small pieces of tissue from 8 and 12 day lesions of fowl-pox were fixed in neutral formalin and absolute alcohol mixture (1 part of neutral formalin to 9 parts of absolute alcohol) for approximately 24 hours.* They were then dehydrated in several changes of absolute alcohol and embedded in paraffin. Serial sections were cut 4 or 5 microns in thickness and alternate ones were mounted on slides, using absolute alcohol as a floating medium and avoiding contact

* This material was obtained through the courtesy of Dr. C. Eugene Woodruff of Vanderbilt University.

A HISTOCHEMICAL STUDY BY MICROINCINERATION OF THE INCLUSION BODY OF FOWL-POX *

W. B. C. DANKS, M.R.C.V.S.

ROCKEFELLER FOUNDATION FELLOW

*(From the Anatomical Laboratory, Washington University School of Medicine,
St. Louis, Missouri) †*

The inclusion bodies occurring in the epithelial cells of fowls injected with fowl-pox virus have attracted the attention of many investigators since their discovery by Bollinger.¹ Previous to 1900 many authors believed them to be microorganisms or parasitic protozoa. Later this hypothesis gave way to the theory that they were products of cellular degeneration due to the action of the virus.

One of the most important of the earlier contributions was that of Borrel,² who showed that smear preparations from the lesions revealed numerous, extremely small, coccoid-like bodies. Burnet,³ two years later, confirmed this work and showed that in stained sections from the lesions these minute bodies and the inclusions occurred in the same cell. These findings led to the belief that the "Borrel bodies" were the causal microorganisms bound up within the cellular inclusions. This theory was upheld by many other authors but some still considered the inclusions (Bollinger bodies) to be simply a product of cellular degeneration. Among those who have supported the latter view are Ludford and Findlay,⁴ whose interpretations, based upon a very detailed cytological study, were that the "Bollinger bodies" were a product of the reaction of the cell to the virus, rather than stages in the life cycle of an actual organism. They showed that the earliest stage in the formation of the inclusion bodies was a small vacuole, to the periphery of which minute granules were attached. The vacuoles then increased in number and size and in many instances coalesced to form one large inclusion body. These virus vacuoles, they pointed out, were enveloped by a lipoid sac and had an internal granular appearance. They further demonstrated the changes occurring in the mitochondria, which usually became

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extent revealed by the color of the oxides to which the inorganic constituents are reduced; free iron is represented by a reddish ash; calcium, along with other salts such as magnesium, leaves a grayish white ash; and organically bound iron, a distinctly yellow residue. The "Borrel bodies," therefore, presumably contain a large amount of calcium, as in the incinerated inclusions they are represented by minute particles which are grayish white in color.

DISCUSSION

It is interesting that the findings in the Negri body and in the fowl-pox inclusion are the same in so far as there is a definite inorganic residue in both following incineration at high temperatures. This would suggest that the "Borrel bodies," like the Negri body, are perhaps products of degeneration of certain cell constituents, due to the action of the virus. It would appear from the results obtained by Woodruff and Goodpasture, who demonstrated the infectivity of "Borrel bodies," that the virus is so strongly combined with the inclusions that tryptic digestion and subsequent washings do not interfere with the union to any marked extent. This hypothesis is not unlikely, as it can be assumed that the "Borrel bodies" by virtue of their inorganic constituents might form a suitable substance for the adsorption of virus. That this is a possibility in the case of the Negri body and other inclusions is still to be proved by employing the technique of Woodruff and Goodpasture.

The incinerated sections of normal fowl skin show that the epithelium is rich in mineral ash, of which calcium forms a large part. The nuclei of normal nerve cells consistently show a small quantity of light brownish yellow ash, indicating the presence of masked iron; peculiarly enough, the nuclei of epidermal cells vary considerably in their visible content of this element. In sections of human skin, for example, the nuclei of some cells are devoid of visible iron while others adjacent to them possess it in relatively large amounts. With the available material it was impossible to determine whether the same holds true for the nuclei of normal skin cells of the fowl. It is conceivable that if a fundamental difference in chemical make-up exists in the nuclei, and perhaps also in the cytoplasm of epidermal cells, this might account for the affinity of certain cells for virus.

Although it can be seen that the nuclei of cells containing fowl-pox inclusion bodies show a decided decrease of inorganic salts, it could

with water. The excess of absolute alcohol was poured off and the sections allowed to dry. The slides were then placed one by one in an electric quartz tube oven and heated through a range of temperatures from about 40°C to 525°C for 25 minutes and, finally, gradually increased to 604°C over a period of 10 minutes. The slides were removed from the oven and cooled slowly. In order to protect the ashed remains coverslips were placed over them and the edges sealed with paraffin. The sections were then examined by means of a Zeiss cardioid condenser. The remaining sections of the series were mounted and colored with erythrosin-azur; these served as controls.

As can be seen from Figure 1, the control sections showed typical fowl-pox inclusion bodies. The lesions were quite far advanced, most of the affected epithelial cells were swollen and in some the nuclei had either disappeared or were represented by a small basophilic staining body at one side of the cell. Other less affected cells revealed the nucleus and the nucleolus quite clearly. Examination of a similar area of the incinerated sections demonstrated an inorganic residue in the inclusion bodies. The refraction of light from the deposits of mineral salts, both in the tissues and in the inclusions, made it impossible to photograph successfully details easily visible by direct examination of the preparations.

Under high power (oil immersion) the control sections, colored with erythrosin-azur, showed typical bodies, many of which consisted of a pink-staining outer area surrounding a clear irregular central area (see Fig. 2). This appearance was helpful in locating the inclusions in the incinerated tissues, as can be seen in Figure 3.

Cells of normal skin which have been incinerated show a considerable quantity of evenly distributed ash. The nucleus contains more mineral than the surrounding cytoplasm and stands out distinctly in the dark-field picture. By contrast the pathological cells show a notable decrease in the amount of both nuclear and cytoplasmic residue. The inclusion bodies themselves consist of a large aggregation of minute particles of grayish white ash (Fig. 4). Many cells show inclusions with this collection of small particles of ash around mineral-free, irregular central areas. This is depicted in Figure 3. Unfortunately the individuality of the particles of inorganic residue is obscured by refraction. These small particles of mineral ash apparently correspond with what are termed "Borrel bodies" both in relative size and in location. The nature of this ash is to some

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DESCRIPTION OF PLATE

PLATE 114

- FIG. 1. Control section of skin lesions from chicken showing numerous fowl-pox inclusion bodies (erythrosin-azur). $\times 300$.
- FIG. 2. Section from the same portion of tissue as Fig. 1, showing inclusions under high power. $\times 750$.
- FIG. 3. Incinerated section showing typical inclusion bodies (a) lying within the cell boundaries (b). Dark-field illumination. $\times 750$.
- FIG. 4. Incinerated section under oil-immersion lens showing a cell containing a circular inclusion body (a) which reveals the particles of ash representing the residue of incinerated "Borrel bodies." The faintly outlined residue of the cell membrane (b) encloses the remains of the nucleus which is slightly to the left of the inclusion body (a). Dark-field illumination. $\times 750$.

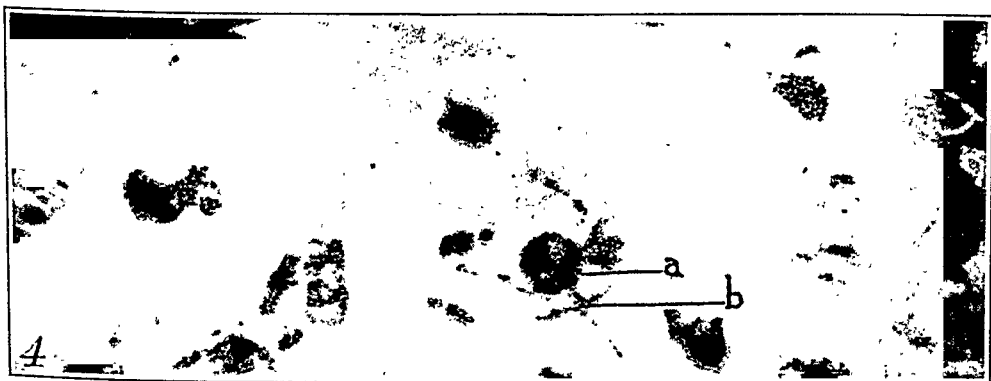
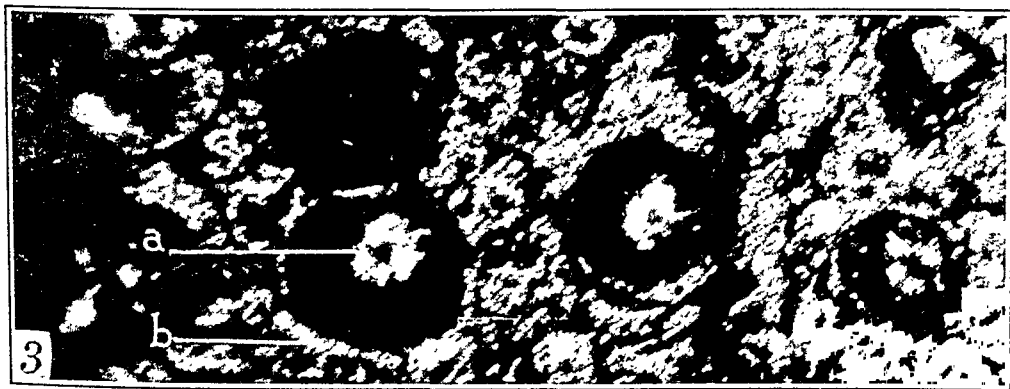
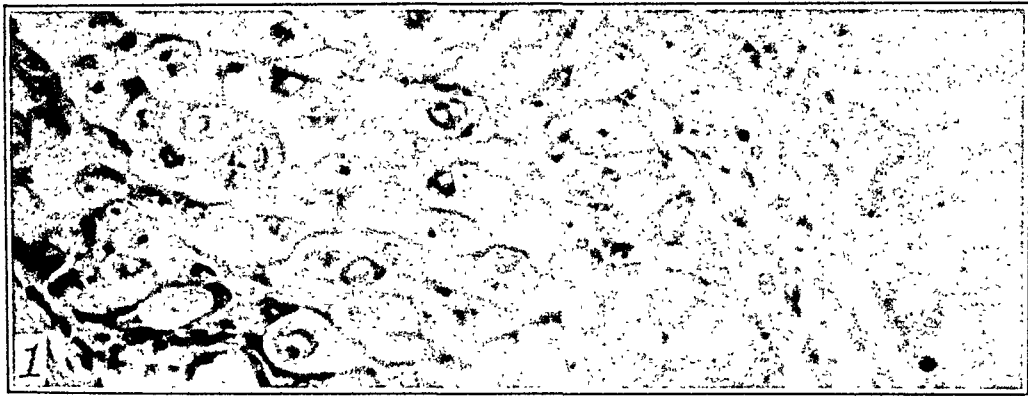
not be concluded that this was the origin of the material forming the inclusion, as has been suggested for the Negri body. It is worthy of note, however, that these observations support those of Ludford and Findlay with regard to the extrusion of chromatin from the nucleus.

In 1930 Scott observed that the nuclear inclusion produced by virus of the submaxillary gland disease of guinea pigs left little or no ash after incineration. In view of these findings it is not entirely inconceivable that the nuclear inclusion represents a chemically different type of degenerative reaction than the cytoplasmic inclusion. Indeed, incinerated specimens of submaxillary glands of guinea pigs, possessing in the same cell both nuclear and cytoplasmic inclusions, do show such a difference. In this instance the cytoplasmic inclusions react the same as the Negri body and the fowl-pox inclusion — they leave a distinct grayish white ash residue after incineration.

There is in all probability a similarity in the nature of the shift of the mineral ash content of the nucleus and of the cytoplasm in nerve cells containing Negri bodies and in epithelial cells which have fowl-pox inclusions. Presumably this is due to the action of virus. With the evidence at hand it is impossible to suggest which cell constituents are responsible for the formation of fowl-pox inclusion bodies, although it is entirely probable that they are degenerative products of cell constituents — a result of the action of virus.

CONCLUSIONS

1. Following incineration at high temperatures the fowl-pox inclusion body leaves a grayish white residue consisting of minute particles of mineral ash. The location of this residue corresponds topographically to that part of the inclusion body which stains pink with erythrosin-azur.
2. The minute particles of mineral ash correspond in relative size and location to the "Borrel bodies" and are the inorganic residue of these structures.
3. There is evidence that the "Borrel bodies" having, as they do, a relatively large amount of inorganic material in them might well serve as a locus for adsorption of virus.



Neubürger.¹⁶ Another case described by Orsós-Debrecen¹⁷ probably should be included in this group.* The necessity of preparing many sections from the region of the rupture was stressed especially by Erdheim, and in the two cases reported by Levinson the changes were so insignificant that they would have been missed in the course of a casual examination.

Recently three cases of spontaneous aortic rupture, without demonstrable inflammatory changes or significant intimal sclerosis at the site of the tear, have been observed in this laboratory. The general histological examination was made from sections stained with hematoxylin and eosin. The elastic elements were stained by Weigert's and Saphir's orcein methods and by a combination of the Verhoeff and the Masson trichrome light green stains. Smooth muscle was identified with the Van Gieson and the Masson trichrome stains. Fat was stained by Sudan III, scharlach R, Nile-blue sulphate and osmic acid. Calcium was demonstrated by the von Kossa test. Chromatropic material was stained by thionin, cresyl violet R, and polychrome methylene blue.

CASE REPORTS

CASE 1. † *Clinical History:* A white man, 50 years of age, collapsed while at rest and died before reaching the hospital. There had been no significant previous illness and his collapse had not been preceded by any unusual physical exertion. The clinical impression was "death from cerebral hemorrhage." The blood pressure was not known. No Wassermann had been taken.

An autopsy was performed and the pathological diagnoses were: rupture of the aorta with dissecting aneurysm of the ascending arch and perforation into the pericardium; hemopericardium (about 500 cc.); cardiac hypertrophy; arterial and arteriolar nephrosclerosis, severe; generalized arteriosclerosis; passive hyperemia of spleen and liver; pulmonary emphysema, severe.

Immediately above the left coronary and non-coronary cusps there was a transverse tear, 4 cm. in length, through intima and media. In association with this tear there was a dissecting aneurysm

* Just before receiving the printer's proof of this study, an article by Klotz and Simpson on spontaneous rupture of the aorta was published. They have described five cases of spontaneous rupture of aortas, the seat of patchy or diffuse medial necrosis, not accompanied by inflammatory reaction or intimal change at the site of rupture. They did not, however, describe fatty change as being a feature of the medial degeneration.

Klotz, O., and Simpson, W. Spontaneous rupture of the aorta. *Am. J. M. Sc.*, 1932, 184, 455.

† The author is indebted to Dr. Harry Goldblatt for permission to publish this case.

MEDIONECROSIS AORTAE IDIOPATHICA CYSTICA *

ALAN RICHARDS MORITZ, M.D.

(From the Institute of Pathology, Western Reserve University, Cleveland, Ohio)

The pathological changes leading to spontaneous rupture of the aorta in the absence of atheromatous or syphilitic lesions at the site of rupture remained vague until Erdheim's^{1, 2} recent description of "Medionecrosis aortae idiopathica cystica." Rupture of the normal aorta incident to severe trauma has been recognized and accepted (Jores³) but the occurrence of spontaneous rupture of a normal aorta has remained a controversial question. Bostroem⁴ was one of the first to describe such an occurrence and to consider the non-traumatic rupture of the normal aorta possible. Jores in reviewing the subject stated: "Spezifische für die Aortenruptur charakteristische Veränderungen sind nicht nachweisbar, ins besondere auch nicht an der Rupturstelle." Kaufmann⁵ cited cases of his own and from the literature of spontaneous rupture in apparently normal aortas. Benda⁶ called attention to the relatively insignificant histological changes seen in cases of spontaneous rupture. Karsner⁷ doubted that a normal aorta is ever the seat of non-traumatic rupture. This latter opinion was shared by von Schnurbein,⁸ who accounted for the non-appearance of demonstrable morphological change on the basis of the angiomalacia described by Thoma,⁹ and by Oppenheim,¹⁰ who tested the resistance of normal arteries to intravascular pressure *in vitro* and found that the pressure required to rupture them was higher than could possibly be attained *in vivo*.

Spontaneous rupture of the aorta has been reported in animals; and Krause¹¹ in a review of vascular disease in animals stated that it is not an uncommon occurrence in horses, and that although arteriosclerosis has been the cause in some, others have been studied in which histological changes were insignificant, or absent.

Erdheim's exhaustive study of two cases of spontaneous rupture of the aorta was prompted by the case reports of Gsell.¹² These ruptures occurred in the ascending arch as the result of medial necrosis, which was distinguished from arteriosclerosis and from syphilis. Additional cases have been reported by Cellina,^{13, 14} Levinson¹⁵ and

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fibrils. This substance was situated between the muscle cells and the elastic fibrils and its presence often caused a swelling of the lamellae. Although the distribution was general, it was more abundant in the middle and inner third of the media. When stained with cresyl violet R and differentiated with dilute acetic acid (Schultz¹⁸) it stained rose or light red. A similar staining reaction was obtained with polychrome methylene blue. The interfibrillar amount of this chromatropic substance varied and with its increase there was a corresponding decrease in the interfibrillar cellular elements. Frequent areas were encountered in which swollen lamellae were identified only by the elastic fibrils, the muscle and connective tissue cells having completely disappeared. When the cellular elements of contiguous lamellae had been replaced by this chromatropic substance, damage to the intervening elastic fibrils was frequently manifest by defects and projecting spurs. When several lamellae had become confluent, cyst formation was observed and a continued accretion of chromatropic substance was suggested by the rounding of the limiting tissue. Such cysts frequently included portions of eight to ten adjacent lamellae and were seen most frequently in the middle and inner thirds of the media. They were not prominent in the immediate vicinity of the tear, and although the largest cysts were found in sections of the ascending portion of the thoracic aorta the chromatropic change and the formation of small cysts were found in the descending portion and in the abdominal aorta. In the abdominal aorta the change was most marked immediately beneath atheromas.

In the ascending thoracic aorta, repair of these defects by fibroblastic proliferation was prominent and various stages of healing were seen up to complete filling in of the defect by fibroblasts. This repair was accomplished without vascularization and at no stage in the process were exudative or phagocytic cells seen. The scars were striking in preparations stained for elastic tissue, because of the sharp delimitation produced by the free ends of elastic fibrils (Fig. 1). The newly formed connective tissue did not conform to the normal structure and the long axis of the cells was frequently oblique or transverse to the long axis of the smooth muscle cells of the aorta. Very fine, newly formed elastic fibrils were seen to form a network in the scars. Such scars were seen in all layers of the media and did not originate in the adventitia.

of the entire ascending portion of the thoracic aorta, so that the separated intima and media lay as a partially detached tube within the adventitia, and separated from it by an extensive hematoma. In the beginning of the transverse arch there was another transverse tear through intima and media for about 1 cm., marking the site of the re-entry of the blood into the lumen of the aorta. Inferiorly the dissecting aneurysm had perforated the pericardium through a ragged defect, measuring about 2 cm. in length. The pericardial sac was distended by about 500 cc. of blood.

The heart weighed 450 gm. and presented no abnormality, other than the hypertrophy which was principally of the left ventricle. The aortic ring measured 7.5 cm. in circumference and the mid-portion of the ascending arch 8.2 cm. The ascending thoracic portion of the aorta was almost completely free from intimal change, there being only a few slightly elevated, irregularly outlined yellow plaques. Moderately severe intimal sclerosis was seen in the lower portion of the thoracic aorta, with increased severity in the abdominal portion.

The media in the region of the tear was thin, but it could not be determined macroscopically whether the thinning was actual, or due to splitting of the media by the dissecting aneurysm. Section through the media disclosed a mottling by small, gray areas, which were in places confluent so as to obscure the entire thickness of the usual yellow zone. No cysts could be identified on macroscopic examination.

Histological Examination: In multiple sections, both transverse and longitudinal, through the aorta at the site of the rupture, the intimal change was minimal. The internal elastic lamella was intact and irregularly thickened. The intima was smooth and exhibited no degenerative changes, other than the presence of homogeneous, faintly staining, basophilic intercellular substance. The subendothelial connective tissue was increased in amount, but not uniformly, so that the free surface was slightly undulating. There was some calcification along the internal elastic lamella and finely dispersed fat droplets in the homogeneous intercellular matrix.

The media was not uniformly thin, and had been split by the dissecting aneurysm which had separated the outside four or five lamellae. Generally throughout the media there was a faintly staining, homogeneous, basophilic substance between and around elastic

and arteriolar nephrosclerosis; infarcts of kidneys; chronic passive hyperemia of lungs, liver, spleen and kidneys; pulmonary emphysema.

There was a transverse tear 2.5 cm. long through media and intima about 1 cm. above the non-coronary aortic cusp. The edges of the tear were sharp and undermined by a dissecting aneurysm which extended about 5 cm. above the tear, and below into the pericardial sac through a slit-like laceration 1 cm. in length. The margin of the tear did not show any evidence of healing and the blood between media and adventitia and within the pericardium showed no organization.

The heart weighed 925 gm. and the hypertrophy was preponderately of the left ventricle. The aortic ring measured 9.5 cm. in circumference and the ascending portion of the thoracic aorta 12 cm. The intima of the ascending thoracic aorta was thin and smooth, except for a few small yellow plaques, the largest being at the commissure between the right and non-coronary aortic cusps, and measuring about 1 cm. in circumference and 2 mm. in thickness. From the arch down, intimal sclerosis became increasingly more severe with atheromatous ulceration of the lower abdominal aorta. Section of the media near the point of rupture did not disclose any characteristic alterations in structure.

Histological Examination: The intima in the region of the tear was irregularly thickened by fibrous connective tissue, but without formation of atheroma. The subendothelial connective tissue was edematous and relatively anuclear. Below the transverse arch intimal sclerosis became more severe, with hyalinization, fat infiltration, formation of atheromatous plaques and ulceration, which extended well into the inner third of the media.

The media in the region of the rupture was thin and profoundly altered in structure. Chromotropic degeneration was focal and severe but cyst formation was not prominent. In the areas of such degeneration, the media appeared attenuated, as though it had stretched in a transverse direction, with elongation of the surviving muscle cells and breaking of the elastic fibrils. In evidence of this, there were entire low power fields in which only short, isolated fragments, or no elastic fibrils were seen. Such defects in the elastic tissue were occupied by parallel-disposed smooth muscle cells, which conformed in direction to the structural pattern of the media

What was interpreted to be a secondary cystic degeneration of partly or completely healed lesions was seen in the form of reaccumulation of serous, rather than chromatropic, substance between fibroblasts, with a disappearance of cells and the reformation of fluid-filled defects (Fig. 1).

There was little, if any, compensatory adventitial thickening for the areas of medial damage. At the site of the tear there was a diffuse extravasation of erythrocytes throughout the adventitia. The vasa showed changes corresponding to those seen generally in arterioles, that is, simple intimal proliferation, without perivascular infiltration. The most severe arteriolar damage was present in the kidneys, where obliteration was frequent. In the adventitia, however, no obliteration was observed and vasa were not uniformly affected, many being unchanged.

No disseminated necrosis of the type described by Cellina was seen either in the aorta or its branches.

Fat was stained in the intima and in the media by scharlach R and Sudan III. In the media it was deposited in the form of small droplets in the chromatropic substance along the elastic fibrils. It was not increased with further destruction of lamellar cellular elements and was not present in the cysts. No anisotropic lipoids were seen with the polarizing microscope.

CASE 2. Clinical History:* A white man, 56 years of age, was admitted to the Cleveland City Hospital with dyspnea, precordial pain and profuse blood-tinged expectoration. The present illness had begun several days previously with a chill and expectoration of blood-tinged sputum, following which his ankles had become swollen. He was known to have had hypertensive heart disease upon a previous admission, at which time his blood pressure was 210/120.

Physical examination disclosed an acutely ill patient with a large active heart, dyspnea, cyanosis and auricular fibrillation. The cardiac embarrassment continued without change until the seventeenth hospital day, when he was seized with a sudden sharp pain in the precordium and left shoulder. He died six hours later with a clinical diagnosis of "probable coronary occlusion."

An autopsy was performed and the pathological diagnoses were: rupture of the aorta with dissecting aneurysm of ascending arch and perforation into pericardium; hemopericardium (1250 cc.); cardiac hypertrophy and dilatation; generalized arteriosclerosis; arterial

* The author is indebted to Dr. David Seecof and Dr. R. W. Scott of the Cleveland City Hospital for permission to publish this case.

The adventitial change was nowhere significant. Vasa vasorum were the seat of some intimal sclerosis, corresponding to the generalized arteriolar disease. In the region of the rupture, and around the hematoma, there was some organization with leukocytic infiltration.

CASE 3. Clinical History: A white man, 44 years of age, collapsed while at work and died five days later. During that period he complained of continuous, intense, precordial pain and his progress was characterized by increasing circulatory failure. The blood pressure and the blood Wassermann reaction were not known. His past history was not significant, other than that he had had rheumatism and influenza thirteen years before. The clinical diagnosis was "probable coronary thrombosis."

An autopsy was performed and the pathological diagnoses were: rupture of the ascending portion of the thoracic aorta with dissecting aneurysm into pericardium; hemopericardium (about 500 cc.); generalized arteriosclerosis, mild; hypertrophy and hyperplasia of thyroid gland.

There was an annular tear through intima and media of the aorta, which included about four-fifths of the circumference of the vessel and was situated about 3.5 cm. above the aortic ring. The maximum separation of the torn edges was 2 cm. Communicating with the tear was a dissecting aneurysm which extended up to the level of the innominate artery and down into the pericardium by means of multiple small lacerations. There was evident organization of the intramural hematoma. Just above the large tear, which had clean, free margins, there was a small parallel tear, the edges of which were approximated by what appeared to be a thin, fibrous cicatrix. The media just above the midportion of the large tear was very thin, and here as well as in other areas near the tear the continuity of media was interrupted by small cystic spaces and gray patches.

Histological Examination: In several longitudinal sections taken through the tear, the external portion of the media above and below it was anuclear and stained lightly with eosin. The junction between this anuclear zone and the adjacent non-necrotic media was sharp and devoid of any reactive changes. The affected tissue had the appearance of coagulation necrosis without exudation. It occupied about half the thickness of the media at the site of the tear and extended about 5 mm. above and below it. The elastic fibers remained unaffected and in sections treated with orcein, without a nuclear counterstain, the lesion could not be recognized. The inter-

(Fig. 2). These cells were separated by chromatropic substance and in consequence the tissue was loose. In other places, exhibiting a similar type of medial defect, the muscle cells were compactly disposed and free from degenerative changes, with occasional nuclei undergoing direct division. The nuclei in such areas were generally larger and more chromatic than in other portions of the media (Fig. 2).

In lesions of this sort the only demonstrable elastic fibrils were either the projecting spurs at the edges or isolated, short, thick fragments of the disrupted fibrils. No new network of elastica was apparent. The defects were principally in the outer third of the media, but in several places they extended completely through the media and included the internal elastic lamella. Their outline generally tended to be wedge-shaped, with the broad base of the defect toward the adventitia; but not infrequently the defect had parallel edges and extended radially, or obliquely from the inner to the outer portion of the media. They were not vascularized and were not associated with exudative cells.

Smaller defects in the media were cystic and apparently due to the confluence of lamellae, the seat of chromatropic degeneration, and in these fibroblastic proliferation was seen to effect repair. These fibroblastic scars differed from the larger defects filled by smooth muscle in that the arrangement of the connective tissue was irregular and without pattern, and in such areas degeneration was usually progressive so that cysts had developed. The contents of the cysts were serous, rather than chromatropic.

The same changes were seen, but with less severity in other portions of the aorta. In the abdominal aorta they were complicated by more severe intimal change, with corresponding medial damage related to it.

The deposition of fat was inconspicuous in the ascending thoracic aorta, and was in the form of fine droplets in the intima and near the elastic fibrils throughout the media. It was not increased in the cysts. In the abdominal aorta, large deposits of fat were observed in the intima and adjacent portions of the media. The fat stained blue with Nile-blue sulphate and red with scharlach R and Sudan III. It did not become impregnated with osmic acid. Finely granular deposits of calcium were demonstrated in the media, particularly in the chromatropic substance, by the von Kossa test.

Calcium was present in the form of finely granular deposits near the internal elastic lamella and occasionally in the chromatropic substance of the media. The presence of fat was prominent in the middle third of the media near the tear (Fig. 3). It was stained red by scharlach R and Sudan III and blue by Nile-blue sulphate, but was not impregnated by osmic acid. The fat was not anisotropic. It was distributed in the form of fine droplets in the chromatropic substance and occasional collections were seen in the poles of the nuclei of muscle cells. In other locations the fat was almost entirely confined to the intima, and here its distribution was scanty and irregular.

The edges of the rupture were covered with fibrin and exhibited organization, especially where the tear extended into the adventitia. The vasa, except immediately beneath the tear, showed no significant pathological change.

DISCUSSION

In the three cases of spontaneous rupture of the aorta just described there were gross and microscopic medial changes which were most severe in the vicinity of the rupture and of such severity as to be considered responsible for the spontaneous tearing of the weakened wall. These changes were not associated with demonstrable inflammation or with significant intimal sclerosis, and in most respects were like those described by Gsell, Erdheim, Cellina, Levinson, and Neubürger in similar cases.

In all three cases there was an increase in homogeneous, pale staining, basophilic, acellular material in the media between the muscle cells and elastic fibrils. In longitudinal section this material appeared to encase the elastic fibrils. This substance was found generally throughout the aorta, but was more prominent in the ascending arch. It stained red with thionin and light red or rose with cresyl violet R and with polychrome methylene blue. It showed a distinct affinity for fat and in one case for calcium. Prolonged formalin fixation rendered the presence of only a small amount of calcium in the other two cases non-significant. The significance and origin of the chromatropic material has been studied exhaustively by Schultz.¹⁸ Because of its property of staining like mucin, a group of investigators, including Hueck,¹⁹ termed the process "Schleimige Degeneration," while Schultz preferred to designate the material as

fibrillar substance stained yellow with the Van Gieson technique and pale green with the Masson trichrome light green stain. This was the only site of this type of necrosis seen in the aorta. It was thought that this necrosis was directly related to the presence of a large intramural hematoma, which lay between adventitia and media, at this point. The hematoma showed extensive peripheral organization, and several vasa in this vicinity were occluded by recent thrombosis. Although the type of necrosis was similar in appearance to the disseminated medial necrosis described by Cellina, the apparent cause for the necrosis was ischemia, due to secondary thrombosis of the vasa incorporated in the large hematoma.

In a number of blocks taken transversely and longitudinally in the vicinity of the rupture, another type of lesion was seen which varied greatly in severity. In one transverse section just above the midportion of the tear only isolated rests of the original media could be identified. These were separated by avascular scars of more or less compactly arranged fibroblasts which frequently involved almost the entire thickness of the media. The scarring was most severe in the mid and outer third, and was not associated with exudation. The scars were not flame-shaped, as seen in syphilitic aortitis, the internal elastic lamella was intact and the overlying intima thin and unchanged.

Chromatropic substance was seen in varying amounts in the media of the aorta and of the pulmonary artery, but was most prominent in the ascending thoracic portion near the rupture. Generally its distribution was in the intima, and between muscle cells and elastic fibers of the media. In longitudinal section it appeared to encase the elastic fibrils. Near the rupture it had frequently replaced the muscle cells of one or several adjacent lamellae with frequent destruction of the intervening elastic fibrils to produce cystic defects. In the larger defects, one of which measured about 2 mm. in length by 1 mm. in thickness and whose transverse extent was not determined, the contents no longer stained specifically for chromatropic substance, but were serous. One such defect was very close to the tear and was filled with extravasated erythrocytes. Spurs and fragments of the original elastic fibrils projected into the defects and in those showing fibroblastic repair a newly formed fine mesh of elastic fibrils was seen. The largest cysts were seen in the middle and outer third of the media, although smaller ones were present in the inner third (Fig. 4).

but was not impregnated by osmic acid. The fat was not entirely extracellular but was seen within the muscle cells, especially at the poles of the nuclei. Finely granular deposits of calcium could be demonstrated in the chromatropic substance by the von Kossa reaction, but as in the case of fat, it too disappeared in the cysts whose contents were serous.

These observations indicated that with age, the aortic media acquires gradually increasing amounts of perielastic chromatropic substance, as described in detail by Schultz, and that the medial change is not necessarily paralleled by equally severe intimal change. Furthermore, this degeneration may result in the formation of circumscribed medial defects, which in the case of spontaneous aortic rupture may attain macroscopic dimensions and apparently produce significant weakening of the aortic wall.

Damage of this sort to the media, without cyst formation, was seen especially in Case 2. Here, in focal areas of lamellar degeneration, the elastic fibers had torn and separated, leaving large portions of the media, sometimes involving its entire thickness, devoid of elastic tissue. In these areas degeneration was in places reversible, because smooth muscle hyperplasia occurred in attempted compensation for the weakening, and advanced lesions occupied by thickly disposed smooth muscle cells were often devoid of degenerative changes (Fig. 2).

The changes so far described have been qualitatively similar in cases of simple aortic sclerosis to those seen in spontaneous rupture. One phase of the process was, so far as our observations are concerned, peculiar to spontaneous rupture, and that was the healing of the foci of medial necrosis. The healing lesions have been described in great detail by Erdheim, whose observations were in accord with ours, except that we did not see regeneration of smooth muscle in the repair of cysts. Hyperplasia of smooth muscle cells was seen at the site of elastic tears (Fig. 2), but in our cases of spontaneous rupture it appeared that the cysts were repaired principally by fibroblastic proliferation (Fig. 1). This repair was accomplished without vascularization and without the presence of exudative cells. The scars marking the site of cyst formation differed from the defects created by elastic tearing in that in the former the pattern of the proliferated fibroblasts was irregular, while in the latter the

a chromatropic substance without definite commitment as to its mode of origin. It is quite well agreed that this chromatropic matrix becomes more prominent in the intima and media of blood vessels with advancing age, and that it exhibits a definite affinity for fat and calcium. Whether it be physiological or pathological, it was regarded by Schultz as "the precursor of further degenerative processes."

Transverse and longitudinal sections of the aortas of seventy adults were studied as to the distribution of the chromatropic substance in individuals not dying of aortic rupture. The relative distribution in intima and media varied. In some it was inconspicuous in the media and prominent in the intima, and *vice versa*. In six of the seventy cases there were focal areas within the media where the entire interfibrillar substance of several contiguous lamellae was acellular and chromatropic, with disruption of the interposed elastic fibrils. Whether the discontinuity of the elastic tissue was artefact, as proposed by MacCallum,²⁰ or actual and indicative of angiomalacia as suggested by the work of Thoma,⁹ it remains that medial defects were seen. In three of the six this medial damage was immediately beneath areas of atheromatous intimal change, and in the other three the medial lesions were not associated with more than mild overlying intimal sclerosis (Fig. 5).

Precisely the same sort of change that was seen in slightly less than 10 per cent of adult aortas, in which only two blocks from each aorta were studied, was present in the three cases of spontaneous rupture, but with greater severity. The medial damage varied from simple accumulation of chromatropic substance around the elastic fibrils to the formation of large, macroscopic cysts (Fig. 4). In the earlier stages the elastic fibrils remained intact while, as the lesion became larger, there was complete destruction of elastic fibrils, leaving short projecting spurs and isolated fragments. These defects were independent of atheroma, and although they were present in all portions of the media the largest cysts were seen in the middle and outer thirds. As the cystic character became manifest, the chromatropic substance lost its specific staining qualities and became serous. Whereas fat in the form of small droplets was found in the interstices between muscle cells and elastic fibers in the earlier stages of degeneration (Fig. 5), it was notably absent from the cysts. The fat stained with Sudan III, scharlach R and Nile-blue sulphate,

curred within a few hours. The longest survival was five days. In all cases the direct cause of death was cardiac tamponade, due to hemopericardium.

In all cases so far reported there has been cardiac hypertrophy, and in three of these, valvular heart disease. In five cases there was chronic renal disease in three of which there was a known clinical hypertension.

Sclerosis of large and medium-sized arteries was a common finding, but not out of proportion to the age of the individual. Diffuse vascular disease with arteriolar changes was seen in at least three cases and possibly more in which the descriptions were not explicit in this regard.

In the cases presented in this report, medial necrosis was most severe in the region of the rupture, but was not limited to that site. Tearing of elastic fibers and cystic degeneration was seen in the abdominal aorta of two of them.

The site of rupture in all of the cases so far reported was in the ascending arch just above the semilunar valves, and the direction of the tear was characteristically transverse. This is in accord with the observations on cases of spontaneous aortic rupture from any cause; and von Schnurbein⁸ in a review of ninety-one cases found that in fifty-six of these the rupture was at this site, and that of the ninety-one cases the tear was transverse eighty-two times. The reason for this particular location of spontaneous rupture, as given by Oppenheim,¹⁰ is that the ascending thoracic aorta has the greatest circumference and that since bursting tension is in direct proportion to the radius of the vessel, the smaller vessels can withstand higher pressure.

In this connection an investigation of the resistance of normal arteries of rabbits to intravascular pressure was made. Six young adult rabbits were used and each was given 20 mg. of heparin per kilogram body weight before the experiment to prevent clotting of blood. The aorta was cut just above the valve under ether anesthesia. The proximal end was ligated and into the distal end a cannula was tied and connected with a pressure bottle containing an india ink-saline injection mixture. The pressure within the injection system was controlled by means of a mercury manometer, which was connected directly with the cannula. The injection of fluid under high pressure was made into the living animal as rapidly as

long axis of the smooth muscle cells conformed to the direction of the lamellae. As pointed out by Erdheim, the degenerative changes and cyst formation constitute a vicious cycle, which he likened to atrophic cirrhosis of the liver. The newly formed media after and during repair underwent degeneration with reformation of cystic spaces. The accumulation of chromatropic material was not a feature of the degeneration in new media, nor was fat infiltration or calcium deposition.

As has been stressed by Gsell, Erdheim, Cellina, Levinson and Neubürger, this type of medial necrosis can in all stages be differentiated from syphilitic mesaortitis. Significant vascular changes in the adventitia fail and there is no exudative reaction. In all of the cases so far described the medial changes were not part of an atherosclerotic process, the intima in the involved region being relatively free from change (Fig. 4).

The disseminated, non-cystic form of medial necrosis described by Cellina was not seen in these cases, although a secondary necrosis near the rupture in Case 3 stimulated this type of necrosis. Here, however, the necrosis was apparently due to secondary occlusion of the vasa vasorum by thrombosis, incident to the dissecting aneurysm. Cellina found this type of necrosis in nine of ten aortas of aged individuals examined. In these nine aortas he found 103 foci of acellular necrosis in which the elastic fibers were preserved. This same type of necrosis was observed by Erdheim and by Levinson in cases of idiopathic medial necrosis, and its significance is still questionable.

The greatest age incidence of idiopathic cystic necrosis of the aorta in all cases so far reported is in the sixth decade, and they ranged from 25 to 83 years of age. Of the twelve acceptable cases in the literature, including the three described in this publication, seven were in men and five in women. In six instances the individuals were known to be at rest when the fatal rupture occurred, and only two were engaged in physical exertion at the time of the fatal seizure. The exact circumstances of the rupture in the other cases was either not known or believed not to be related to any unusual exertion. The initial seizure was known to be accompanied by pain in three instances, substernal in two and upper abdominal in one. The survival following the seizure varied, but in most instances death oc-

much greater since these were found in the course of a study of routine sections. It is true that the cysts were small, rarely involving more than three or four contiguous lamellae, and healing of such defects was not seen, but the differences between these defects and the lesions found in cases of spontaneous rupture were quantitative rather than qualitative. Without subscribing to Thoma's opinion as to the relation of medial weakening to atherosclerosis, it seems possible that the medial necrosis described in sclerotic aortas without rupture, as well as in the vicinity of spontaneous tears, is in accord with Thoma's idea of angiomalacia. This does not detract from the suitability of Erdheim's term "*Medionecrosis aortae idiopathica cystica*," but does throw a different light upon its significance.

SUMMARY AND CONCLUSIONS

Three cases of spontaneous rupture of aorta with cystic necrosis of the media have been described. The necrosis developed focally in areas the seat of chromatropic or mucinous degeneration, and was not associated with significant intimal change or inflammatory reaction. These have been compared with the previously reported cases of this disease and certain additional observations made. Tearing of the elastic elements occurred with and without cystic change and neither the cystic change nor the elastic tears were limited to the ascending arch of the aorta. The lesions were qualitatively similar to those commonly seen in sclerotic aortas, and differed in that the necrotic foci were larger and exhibited a greater tendency to repair. In the cases reported to date evidence of hypertension has been common but not constant, and in a considerable number the rupture has occurred while the individuals were at rest. Additional case studies will be profitable in establishing the similarity or dissimilarity of this disease to the changes commonly seen in arteriosclerosis without advanced intimal lesions.

possible, so that within two or three minutes after cutting the aorta intra-aortic pressures of 800 mm. of mercury or more were obtained. It was recognized that such pressures were not maintained at any considerable distance from the cannula, because of their rapid dissipation by flow of fluid into the veins. Loss of blood from the operative wound was prevented by overlapping Kelley clamps.

In all six rabbits, a sudden fall in pressure was noted after a pressure ranging between 800 and 1200 mm. of mercury had been obtained. This fall in pressure was followed by abdominal distention and rapid exhaustion of the injection mixture. The abdomen was opened, and by immersing the rabbit in running water the point of vascular rupture could be identified. In all six rabbits the rupture was either in the portal vein or in one of its primary tributaries. It was not possible in these experiments to rupture the arteries because of the rapid transfer of sufficiently high pressures to the veins to rupture them.

Macroscopic changes deemed characteristic were described by Erdheim and consisted of transverse, parallel-disposed, gray scars, marking the site of medial damage, beneath a thin, non-altered intima. Cyst formation within the media also reached macroscopic proportions.

The pathogenesis of the lesions resulting in the rupture appears to be quite definite so far as morphology is concerned. The etiology remains obscure, and we agree with Erdheim that it will be necessary to study many such cases to appreciate the conditions under which this peculiar type of necrosis develops. Erdheim has compared and distinguished these lesions from the medial changes seen in postinfectious cases by Wiesel,²¹ from the postinfluenzal vascular damage described by Stoerk and Epstein,²² and rheumatic arteritis by Pappenheimer and VonGlahn.²³ He has raised the question of avitaminosis or adrenalin poisoning as possible etiological agents.

This study suggests the probability that the disease is involutional or senescent in character. The damage results from the excess accumulation of chromatropic substance or mucin-like material, described by Schultz as representing an aging phenomenon in the arteries of animals and of men. Small cystic defects of the media were present in slightly less than 10 per cent of the sclerotic aortas of adults examined and were not necessarily identified with overlying atheromatous change (Fig. 5). The actual percentage may be

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DESCRIPTION OF PLATES

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FIG. 1. (a) Hematoxylin and eosin and (b) orcein stains of same area in outer-third of media in two successive sections of same block from aorta of Case 1. An area of medial degeneration appears almost entirely healed in (a), but the extent of the elastic damage filled in by fibroblasts which do not conform to the lamellar structure of the media is seen in (b). Secondary degeneration has taken place in the repair tissue. $\times 124$.

FIG. 2. Midportion of media of aorta from Case 2 near rupture. Chromotropic degeneration with cyst formation is seen in upper portion of photograph, while in lower portion the defect in the elastica is bridged by hyperplastic smooth muscle cells, the long axis of which is parallel to the elastic fibrils. The block was taken near the tear and extravasated erythrocytes are seen in the small cyst. Stained by orcein, according to Saphir. $\times 124$.

FIG. 3. Midportion of media of aorta from Case 3. Stained by Sudan III to show fat droplets in the chromotropic substance between elastic fibrils and muscle cells near rupture. Wratten (C) filter. $\times 320$.

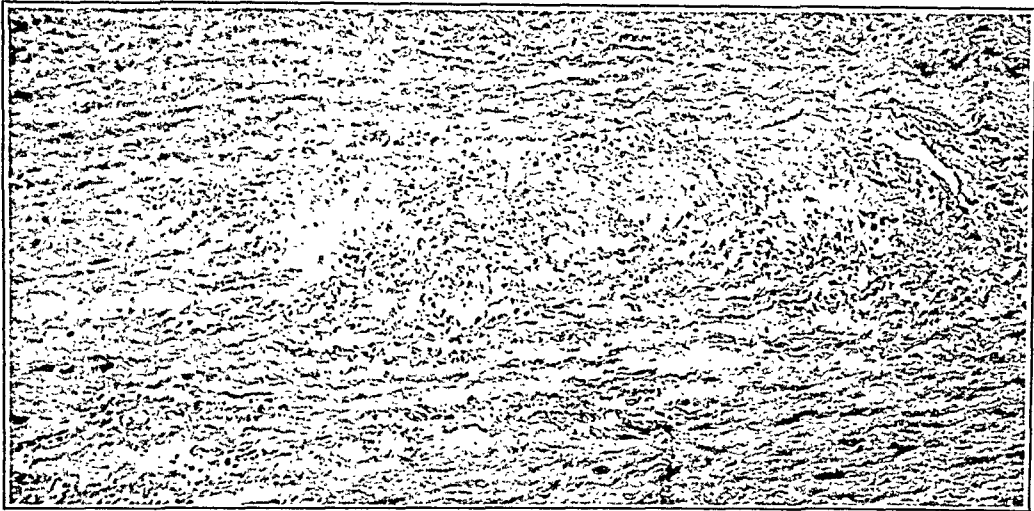
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PLATE 116

FIG. 4. The entire thickness of intima and media of aorta from Case 3, taken near rupture and including large cyst in midportion of media. Disruption of elastic fibrils is seen in the outer third with proliferation of fibrous connective tissue to repair defect. The intima is free from sclerosis, the internal elastic lamella is intact and there are no exudative cells. Stained by combination of the Verhoeff elastic and the Masson trichrome light green methods. $\times 75$.

FIG. 5. (a) Hematoxylin and eosin and (b) orcein stains of same section of aorta of a 34 year old man who died of pneumonia. No syphilis and minimal intimal sclerosis. Focal increase of chromatropic substance with confluence of lamellae due to disruption of elastic fibrils. Probable precursor of more severe changes seen in "Medionecrosis aortae idiopathica cystica." $\times 134$.



1 (a)



1 (b)



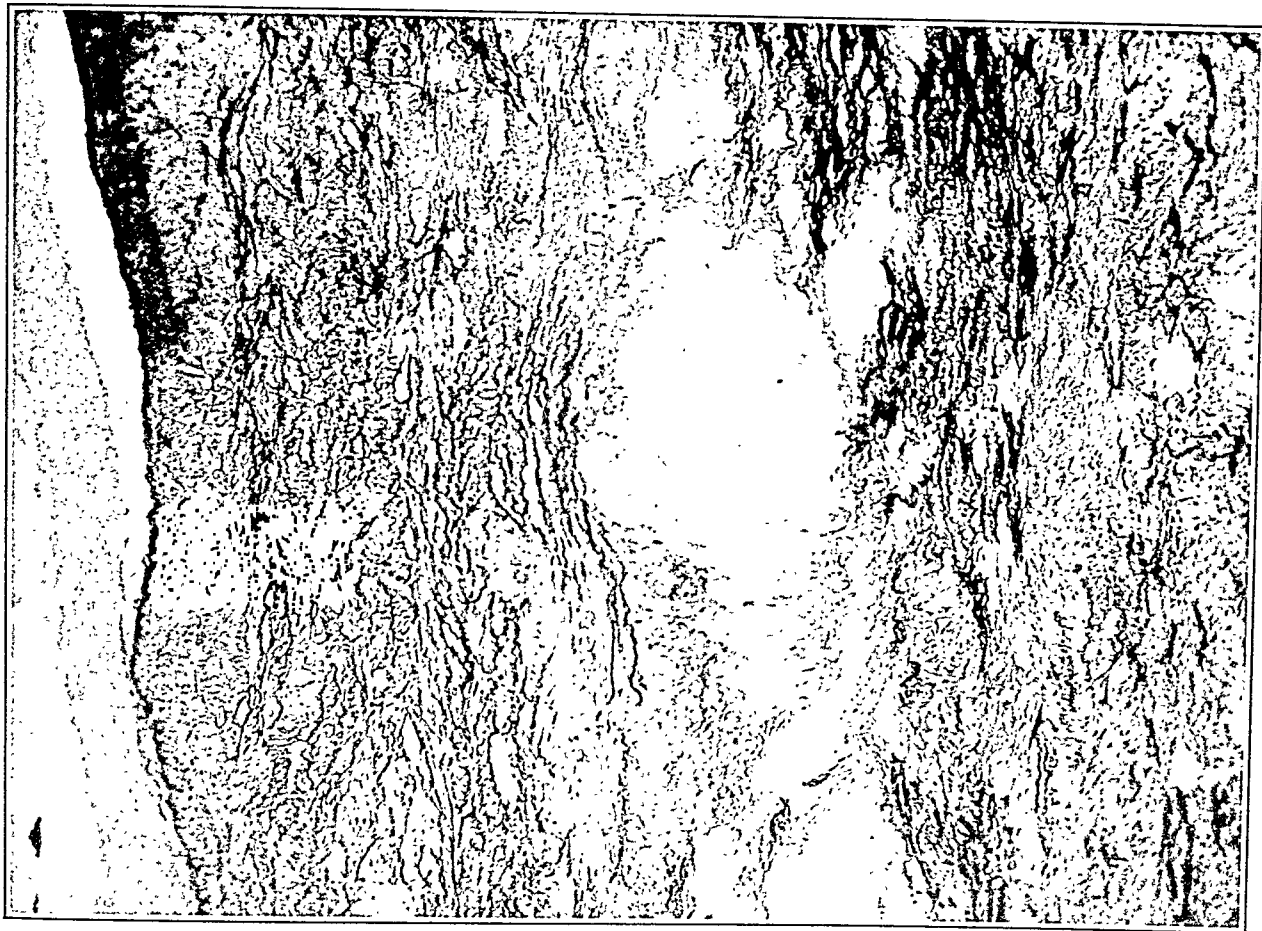
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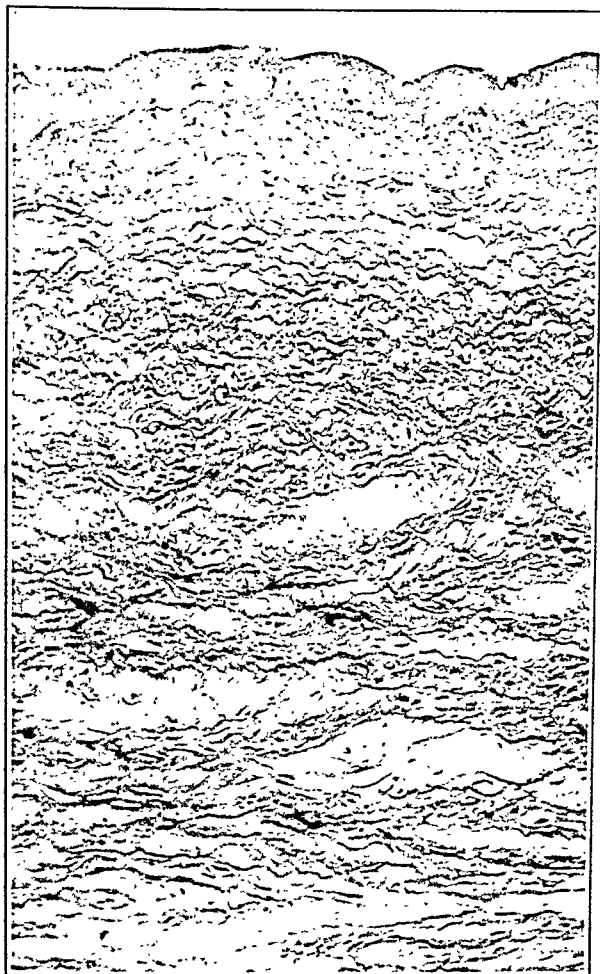


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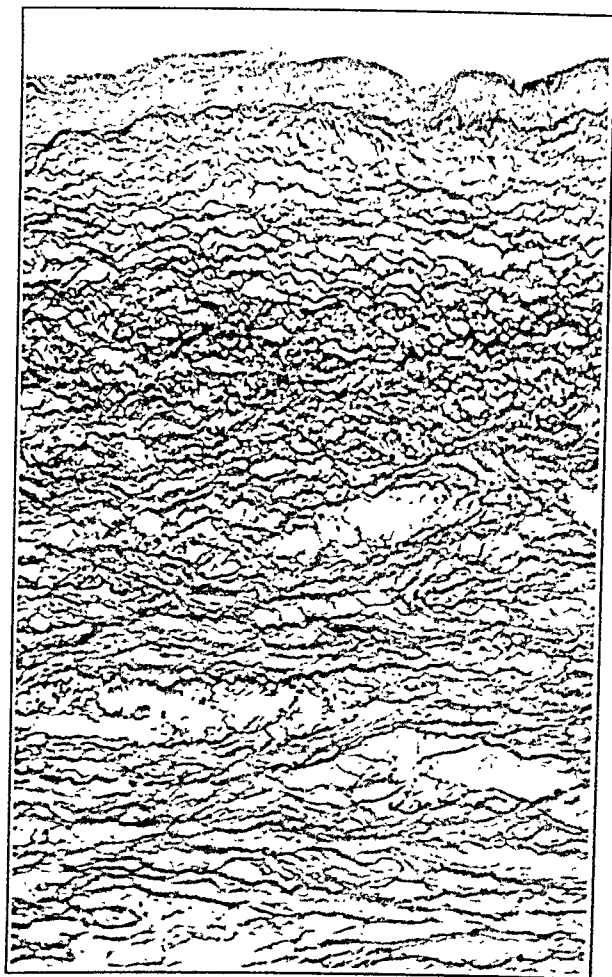
Medionecrosis Aortae Idiopathica Cystica



4



5 (a)



5 (b)

has not been restricted arbitrarily to conditions so completely primitive, but has been applied to the suspension of the small intestine from a midline pedicle affording great mobility to the small intestine and proximal portion of the colon (Anders³).

Anders, in a comprehensive review of congenital malformations of the intestine and mesentery, states that a "mesenterium commune" exists in all cases in which there has been complete or advanced retardation of intestinal rotation. The correctness of his conclusion seems apparent, since proper fixation would depend upon proper position, but it does not follow that proper rotation would always predicate normal secondary attachments. It seems likely that regardless of the manner in which the intestine rotates in its return to the abdominal cavity the hypermobility permitted by a "mesenterium commune" may lead to a wide variation in the position observed in postfetal life. Dott,⁴ and Haymond and Dragstedt⁵ have reviewed the various types of abnormal position and have considered them varying degrees of reversed rotation, rather than of incomplete rotation.

A consideration of the association of "mesenterium commune" with incomplete rotation in a normal direction, as well as with reversed rotation, is of more than academic interest. Many of the cases presented by Dott and by Haymond and Dragstedt are characterized by intestinal obstruction due to volvulus. If the "mesenterium commune" existing in such a case were due to a primary reversed rotation which occurred *in utero*, the obstructing volvulus would have to be corrected in such a manner as to bring the intestine back to its original position of reversed rotation, because a position acquired by the intestine in the tenth week of fetal life would have become, in all probability, normal for that individual.

Recently four cases of "mesenterium commune" have come under our observation, two of which have simulated reversed rotation because of volvulus.

CASE REPORTS

CASE I. MESENTERIUM COMMUNE

Clinical History: A white, female, cretin child, 7 months of age, was admitted to the Babies' and Children's Hospital and died following a respiratory infection without any significant gastro-intestinal complaints.

MESENTERIUM COMMUNE WITH INTESTINAL OBSTRUCTION *

ALAN RICHARDS MORITZ, M.D.

(From the Institute of Pathology, Western Reserve University, Cleveland, Ohio)

Anomalous secondary mesenteric attachments of both small and large intestine are common and present a wide variation in degree and location. An understanding of their pathogenesis rests upon a knowledge of fetal intestinal rotation. The description and definition of the successive stages of intestinal rotation and mesenteric attachment by Frazer and Robbins¹ is generally accepted and anomalies are classified according to that stage of development in which a departure from the normal occurred. A critical period in the development of the intestine is in or about the tenth week of fetal life when the gut returns to the abdominal cavity from the umbilical cord. Normally this return is accomplished in such a manner that the proximal portion of the colon lies in a plane ventral to the small intestine, crossing in front of the terminal portion of the duodenum from right to left. Subsequent rotation is counter clockwise and is followed by the secondary mesenteric attachments characteristic of the normal adult type.

The commoner anomalies of secondary mesenteric fixation are concerned chiefly with incomplete fixation of the ileocolic segment and have been adequately reviewed by Waugh.² One of the more extreme types of variation has been termed "mesenterium commune," which implies an absence of the secondary attachments to such a degree that the original prefixational type of suspension persists, that is, a common mid-dorsally attached mesentery for small and large intestine. The term "mesenterium commune," however,

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Dr. W. E. Ladd has described three cases of mesenterium commune with volvulus and intestinal obstruction in a published study (Ladd, W. E. Congenital obstruction of the duodenum in children. *New England J. Med.*, 1932, 206, 277), and has been so kind as to permit the inclusion in this report of a fourth case which has not been published (personal communication). These were cases of clockwise volvulus of the entire jejunum, ileum and proximal half of the colon in children who were operated upon at 2 weeks, 4½ weeks, 9 months and 1 year of age respectively, for obstruction of the third portion of the duodenum. All four children survived the operation with subsequent relief from obstruction. Because so much of the intestine was included in the volvulus Ladd has stressed the necessity of "delivering" the whole small bowel and untwisting it.

bination of traction above and massage below. The intestine appeared viable and in surprisingly good condition, save for edema of the cecum and ascending colon. Silk sutures were employed to fix several inches of the ileum to the wall of the cecum to prevent a recurrence of the intussusception. After reduction of the obstruction the large intestine occupied the left side of the abdomen and the small intestine the right. The large intestine was suspended on a long mesentery and was unusually mobile.

The child died twenty hours after operation with a clinical diagnosis of acute peritonitis.

Postmortem Examination: Twenty centimeters of the lower ileum were hemorrhagic, dilated and edematous. The mucosa was necrotic and the lumen contained sanguineous fluid. There were several silk sutures attaching the ileum to the wall of the cecum. There were 50 cc. of serosanguineous fluid in the abdominal cavity.

As in the preceding case the secondary mesenteric attachments were defective. The base of the mesentery on the posterior abdominal wall followed the general outline of an interrogation point (?), the upper limb starting just below the superior mesenteric artery in the midline from which the line of attachment ascended slightly, then extended to the left side of the abdomen and down to the pelvis in the midline. The mesentery was long, permitting great mobility of both small and large intestine and they could easily be manipulated by rotating the small intestine clockwise on its mesenteric pedicle until the superior mesenteric artery was brought to a position behind the duodenojejunal junction and the colon into a plane posterior to the small intestine. The possibility of this rotation was significant in the understanding of the succeeding two cases.

There were no other significant pathological changes.

Case 3. Mesenterium Commune with Volvulus

Clinical History: A white, male infant, 3 weeks of age, was admitted to the Babies' and Children's Hospital with a history of persistent vomiting. The family history was not significant. There had been no bowel movements from birth although a small amount of green material was passed after enema. The child had taken water and feedings eagerly but had vomited after each feeding. Roentgenological examination after barium by mouth indicated obstruction to the third portion of the duodenum which was not relieved by atropin. The duodenum proximal to the obstruction was dilated and the barium gradually passed the point of obstruction.

The patient was transferred to Lakeside Hospital and operated upon by Dr. J. W. Holloway. The stomach and duodenum were greatly distended and there was a palpable cord-like obstruction in the third portion of the duodenum.

Postmortem Examination: In addition to hypoplasia of the thyroid gland and an acute bronchitis there were defective secondary mesenteric attachments.

On opening the abdomen the cecum was found to be in the epigastrium in the midline, with the colon occupying the left and the small intestine the right side. The greater omentum was undeveloped. The entire jejunum and ileum were suspended from a common mesenteric axis in the midline. The cecum and proximal portion of the colon were suspended by a redundant mesentery which extended upward from the pedicle of attachment of the small intestine and then to the left with descent on the left posterior surface of the abdominal wall toward the pelvis. The configuration of the posterior mesenteric attachment was that of an interrogation point (?), (Text-Fig. 1). The superior mesenteric artery crossed over the third portion of the duodenum in the usual fashion. Because of the hypermobility permitted by the defective attachments it was possible to rotate the small intestine, cecum and proximal portion of the colon through an arc of 360° in a clockwise direction and bring the colon behind the duodenojejunal junction, and by reason of the torsion of the proximal portion of the jejunum the superior mesenteric artery assumed a position between the small and large intestine. Figures 1, 2 and 3 show the successive stages of this manipulation and Figures 4, 5 and 6 are diagrams indicating the relative changes in the relation of small and large intestine and superior mesenteric artery (see legends).

CASE 2. MESENTERIUM COMMUNE WITH INTUSSUSCEPTION

Clinical History: A white, male child, 9 months of age, was admitted to the Babies' and Children's Hospital because of abdominal pain, vomiting and bloody stools of three days duration. The onset of the illness was characterized by vomiting, followed by intermittent paroxysms of pain. The past and family history were negative.

The child was in constant distress with frequent acute attacks of pain. The abdomen was tense, rounded and rigid. There was a large fusiform mass palpable in the left side and extending into the rectum. Peristalsis was active. Laboratory examination was not significant, other than a count of 16,400 white blood cells.

The patient was transferred to Lakeside Hospital for operation by Dr. J. W. Holloway. At operation the cecum was found to be situated to the left of the midline, high in the abdomen, and there was an intussusception of a large part of the small intestine into the colon, the tip of the intussuscepted bowel being palpable in the rectum. The intussusception was completely reduced by a com-

former places, although the condition was still that of a left-sided colon. The duodenum was now behind the superior mesenteric artery.

Case 4. Mesenterium Commune with Volvulus

Clinical History: The patient was a white, male infant, 7 weeks of age, who entered the Babies' and Children's Hospital because of persistent vomiting. Despite the vomiting after each feeding there had been a gain of $1\frac{1}{2}$ pounds since birth and occasional well formed stools had been passed. Roentgenological studies showed the stomach and duodenum to be dilated, with partial obstruction at the duodenojejunal junction. Although most of the barium was vomited, some passed through the large intestine in eighteen hours.

The patient was transferred to Lakeside Hospital and operated upon by Dr. F. S. Gibson. The terminal portion of the duodenum was obstructed by a volvulus of the entire jejunum, ileum and part of the colon. The colon was posterior to the duodenum and so firmly fixed by adhesion that anatomical restitution was not considered possible.

The child died four hours after operation.

Postmortem Examination: The intestinal malposition was essentially that described in the operative note. The mesenteric attachments of the colon were entirely to the left of the midline. The distal portion of the duodenum was mobile, and it, as well as the remainder of the small intestine, was attached by means of a narrow pedicle in the midline which took origin below the region of the superior mesenteric artery and extended inferiorly and to the right of it for a short distance. There had been a twist of 360° in a clockwise direction of the entire jejunum and ileum and proximal colon, so as to bring the colon behind the duodenojejunal junction, where it was fixed by dense fibrous adhesions (see Fig. 4). The superior mesenteric artery was also brought to a position between the duodenum and colon. After freeing the fibrous adhesions the colon and artery were returned to their proper place in relation to the small intestine by rotation through 360° in a counter clockwise direction. The duodenal mucosa at the site of the twist was edematous and hemorrhagic and the muscularis was fibrous. There was no change, other than the serosal thickening, in the wall of the colon where it was fixed behind the duodenum.

DISCUSSION

Four cases presenting defective secondary mesenteric attachments of the intestine of the type commonly called "mesenterium commune" are described. The attachment of the mesentery to the

The cecum was adherent to the anterior surface of the duodenojejunal junction and upon freeing it the colon was seen to pass from right to left behind the twisted segment of duodenum. The colon was fixed in this position by adhesion to the posterior abdominal wall. After the cecum had been dissected free it was possible to demonstrate the patency of the obstructed portion of the duodenum by pressing on the stomach. The operative note is concluded with the statement that "however, one could not establish normal relations by any process of rotation. In view of the fact that the duodenum at least was anatomically patent, it was felt that nothing could be accomplished by further exploration."

The child died on the second day after operation.

Postmortem Examination: Three distinct abnormalities were disclosed by examination of the abdominal contents.

The first anomaly was defective mesenteric attachment, similar to that described in the previous cases in which the distal portion of the duodenum was unusually mobile, the small intestine suspended from a common mesenteric root, the axis of which was the superior mesenteric artery, and the colon suspended on a long mesentery whose posterior attachment followed a line which curved to the left and then descended toward the midline of the pelvis.

The second anomaly consisted of a clockwise volvulus of 360° whereby the duodenum had been drawn anterior to the superior mesenteric artery and the colon posterior to it, with fixation of the colon by fibrous adhesions in that position (see Fig. 4). The twisted segment of duodenum was surrounded by fibrous adhesions but was not completely obstructed. Most of the adhesions had been dissected free at the time of operation, and there was considerable hyperemia and edema at this site. Both on gross and microscopic examination the absence of injury to the muscularis indicated that the twisted segment of the gut had not been severely damaged and that the volvulus had not caused any considerable degree of stenosis.

The third anomaly was an intra-abdominal hernia by which a large part of the small intestine had passed through a defect in the mesentery close to the ileocecal junction. The aperture in the mesentery was about 1 cm. in diameter and the margins of the orifice were slightly thickened. This mesenteric hernia was apparently the condition responsible for the surgical failure to re-establish normal relationship. It was first necessary to reduce the hernia, which was done without difficulty and without dissection. The colon was freed of its secondary adhesion behind the duodenum and the large and small intestine rotated in a counter clockwise direction through an arc of 360° . This returned the large and small intestine to their

artery lay between the small and large intestine (Fig. 4). That this malposition was a volvulus rather than reversed rotation during fetal life, was indicated by the torsion of gut and mesentery at the axis of rotation which was at the duodenojejunal junction. The abnormal mobility of the terminal portion of the duodenum prevented the superior mesenteric artery from being obstructed by being included in the twisted segment. The manipulation required for the relief of the obstruction brought the colon and the superior mesenteric artery anterior to the duodenojejunal junction and in their normal planes. Figure 4 indicates the abnormal relations incident to the volvulus and Figures 5 and 6 show the successive stages in the reduction of the volvulus by a counter clockwise rotation of 360° as indicated by the arrows. The relations obtained by this corrective manipulation (Fig. 6) represent those of normal fetal rotation but with the defective secondary fixation illustrated in Text-Fig. 1.

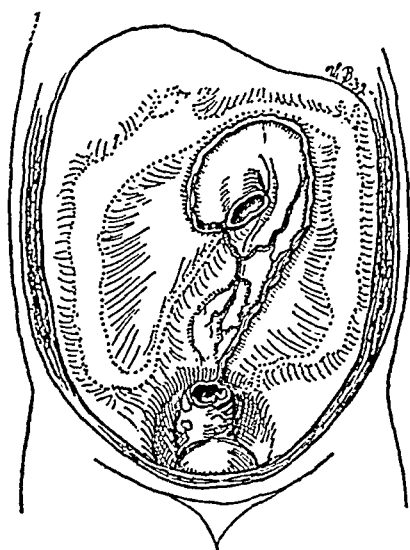
Inasmuch as such conditions are likely to be seen first with a limited surgical exposure it is important to appreciate how closely a volvulus resulting from a "mesenterium commune" may simulate abnormal fetal intestinal rotation. The manipulation required for the relief of a volvulus due to defective mesenteric fixation in an intestine which has rotated normally is obviously quite different from that which would be effective in relieving an obstruction in a malposition of the intestine because of abnormal fetal intestinal rotation. In either instance the intestine would have to be returned to the position normal for that individual, whether that position be the result of the usual or of reversed direction of fetal rotation. Regardless of the direction of fetal rotation the position of the intestine resulting from it would have become normal for the individual, inasmuch as the direction of rotation is established by the end of the tenth week of fetal life and all further growth of intestine and mesentery are conditioned by it.

CONCLUSIONS

Four cases of "mesenterium commune" have been described, one in an infant manifesting no disturbance referable to the anomaly, and three in infants who died of intestinal obstruction secondary to the hypermotility engendered by the defective mesenteric attach-

posterior abdominal wall followed a pattern similar to an interrogation point (?), the small intestine being suspended from a pedicle, beginning at the upper limb of the ? with the line of attachment of the colon, deviating to the left and then descending toward the midline into the pelvis (Text-Fig. 1). In none of the cases was the mesentery of the colon fused with the greater omentum and in all the greater omentum was vestigial.

The first case was of an infant, 7 months of age, in whom the defective mesenteric attachments were an incidental autopsy finding without any clinical record of obstructive phenomena.



TEXT-FIG. 1

Drawing illustrating the dorsal line of mesenteric attachment as seen in all four cases (solid lines) in contrast to the normal secondary line of dorsal attachment that should have been present (dotted lines).

In the second case, which was of an infant, 9 months of age, the defective mesenteric attachments had made possible an intussusception of the small into the large intestine of such magnitude that the advancing end of the intussuscepted gut could be palpated by a rectal examination.

In Case 3 and Case 4, which were of infants, 3 and 7 weeks of age respectively, the condition disclosed at operation and autopsy simulated reversed rotation in the second stage of intestinal development (Frazer and Robbins). The colon was fixed behind the small intestine at the duodenojejunal junction and the superior mesenteric

DESCRIPTION OF PLATE

PLATE 117

FIGS. 1, 2 and 3. Photographs of the successive stages in the reduction of the volvulus seen in Cases 3 and 4. (See Figs. 4, 5 and 6 for interpretation.)

1. Clockwise volvulus of 360° with reversal of the planes of large and small intestine.
2. Reduction of the volvulus by 180° in a counter clockwise direction.
3. Complete reduction of the volvulus with establishment of normal position.

FIGS. 4, 5 and 6. Drawings showing the relations of small and large intestine to one another and to the superior mesenteric artery in the photographs shown in Figs. 1, 2 and 3.

4. Volvulus of 360° as shown in Fig. 1. The arrow indicates the direction of rotation necessary for reduction of the volvulus.
5. Reduction of the volvulus by 180° as shown in Fig. 2. The arrow indicates the direction of rotation for complete reduction.
6. Complete reduction with establishment of normal position.

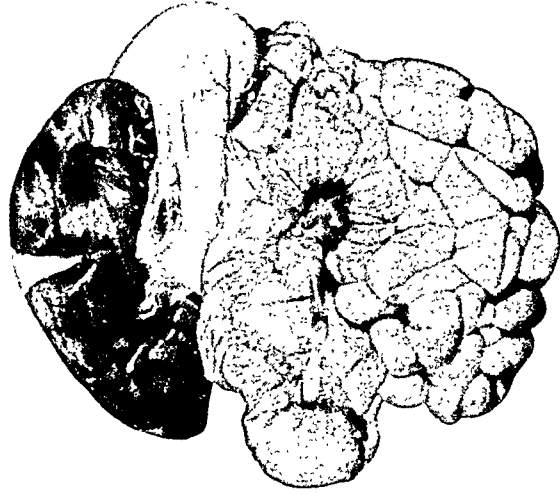
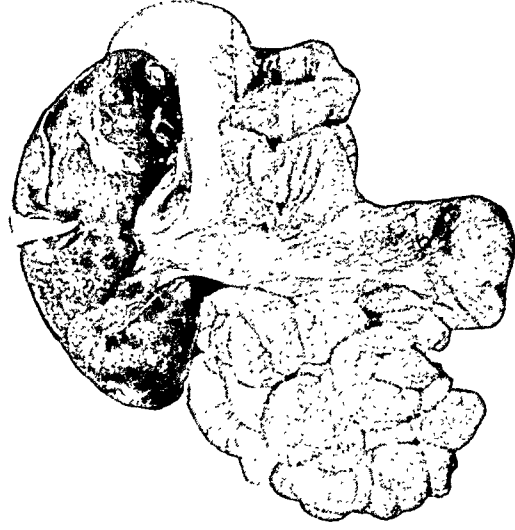
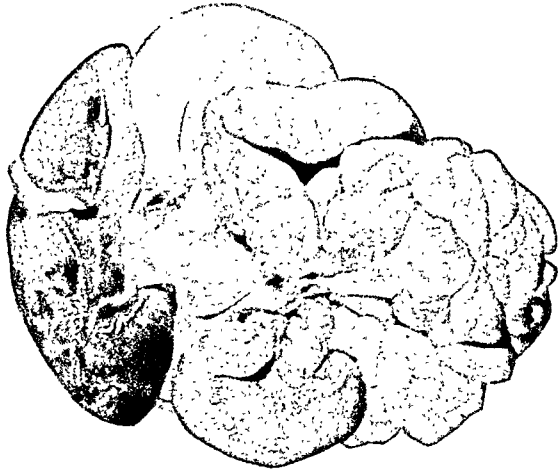
ments. In two of the infants, the obstruction was caused by a volvulus of 360° in a clockwise direction, which because of the abnormal motility of the terminal portion of duodenum reversed not only the planes of the small and large intestine, but also the relation of superior mesenteric artery to the duodenojejunal segment of the intestine. The condition in these two cases was such as to stimulate reversed developmental rotation.

These cases illustrate the surgical necessity of determining at the outset whether the condition is a real reversed rotation or a secondary volvulus due to defective fixation. Correction of a true reversed rotation established during the third month of fetal life must regard the reversal as normal for that individual, while only the volvulus type may be reduced by returning to normal position.

The author wishes to thank Dr. J. W. Holloway, Dr. F. S. Gibson, and the Babies' and Children's Hospital for the use of their records, Miss Theodora Bergsland for the drawings and Dr. B. M. Patten for helpful advice.

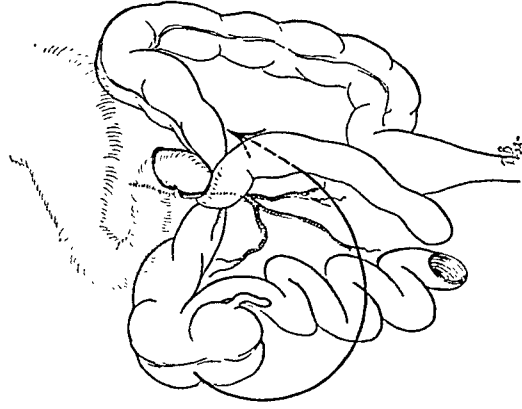
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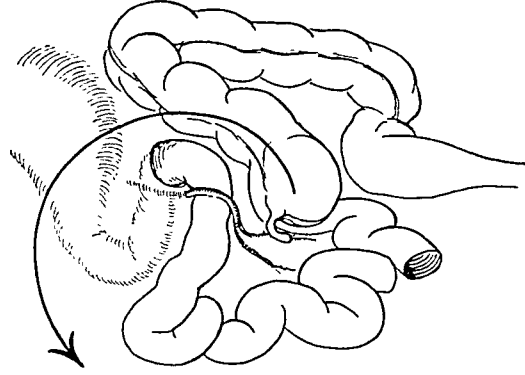


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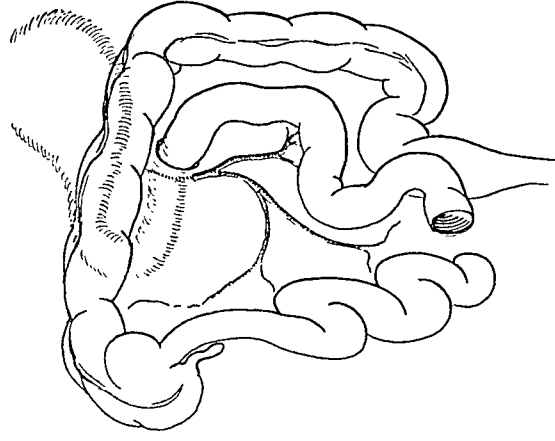
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